THE ELEMENTS

OF

VEGETABLE HISTOLOGY

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BY

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PREFACE TO SECOND EDITION

In this Second Edition the chapters on the chemical reactions of plant tissues, staining, fruit structure and seed structure will be found revised and enlarged. In practice it had become evident that these chapters were in need of amplification. The author has duly accomplished this, while endeavoring, as far as possible, to maintain the plan of condensation and conciseness of statement aimed at from the beginning.

Minor changes have also been made throughout the book in the interest of enhanced usefulness and to bring the work up to date.

The author is indebted to Professors R. M. Holman and W. W. Robbins for permission to use several illustrations from their "Text Book of General Botany" and due acknowledgment is made in the titles of the illustrations reproduced therefrom. Thanks are also due the Bausch & Lomb Optical Company for illustrations of apparatus useful in the laboratory.

<div align="right">C. W. B.</div>

PREFACE TO FIRST EDITION

A KNOWLEDGE of vegetable histology is the foundation for the studies of microscopic pharmacognosy and microanalysis of foods. It is so intimately related to the studies of plant morphology and plant biology that these subjects should be considered prerequisite or should parallel the course in plant histology.

This volume is intended for the beginner; and, in many instances, details have been omitted in order that the student may obtain a knowledge of general principles without being confused by the minutiæ of the subject. Those intending to specialize in vegetable histology, pharmacognosy and microanalysis of foods will find a wealth of detail in the reference books noted in the Appendix.

The amount of laboratory work in vegetable histology performed by the student is necessarily governed by the amount of time at his disposal. Lectures should be abridged if necessary, in order that a greater amount of laboratory work may be accomplished, for in no other branch of science is the personal equation of such importance in the proper interpretation of findings. Microscope equipment for a class should be as nearly uniform as possible in order that the complications due to different magnifications may be eliminated.

The writer would indeed be remiss if he did not

at this opportunity express his appreciation of the influence of Dr. H. H. Rusby, Professor of Materia Medica, College of Pharmacy, Columbia University. Dr. Rusby's labors have been a source of inspiration; and association with him has been responsible for whatever measure of success the writer has attained in the field of microanalysis.

For constructive criticism and generous aid in revision of the manuscript, I am indebted to Fanchon Hart, Instructor in Materia Medica, College of Pharmacy, Columbia University, who by kindly help has greatly expedited the issuance of this manual.

Acknowledgment is also due to the Bausch & Lomb Optical Co., E. Leitz and the Spencer Lens Co., for the use of illustrations and data from their several catalogues.

<div align="right">C. W. B.</div>

Columbia University,
  College of Pharmacy,
    November, 1920.

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THE ELEMENTS OF VEGETABLE HISTOLOGY

CHAPTER I

PREPARATION OF SPECIMENS

THE preliminary operations in the preparation of plant tissues for micro-examination include sectioning and powdering of the material under consideration. Either or both of these procedures may be necessary because plant organs are usually too large and too thick for direct examination. Although much information may be gained from a surface examination by the use of reflected light, it must be remembered that details of inner structure are of the greatest importance in vegetable histology and a knowledge of these details can only be gained through examination of powdered or sectioned materials.

SECTIONING METHODS

The methods employed in the preparation of sections or thin slices of plant parts vary according to the texture of the tissues and the type of section required. In general, the methods may be divided into (*a*) those in which infiltration, or the permeation of the cell with a supporting medium, is necessary and (*b*) those in

which infiltration with a supporting medium is unnecessary.

This method of treating materials for sectioning is used in the preparation of specimens of animal tissues and the finer types of vegetable sections, especially those used to illustrate the protoplasmic contents and the parts of the living cell. In brief, the process consists in killing the tissue without disturbance of the cell contents and involves a replacement of the water in the cells by a supporting medium. The necessary operations include fixation, dehydration, clearing, infiltration, embedding, cutting and staining.

Fixation.—The object of this process is to abruptly terminate the life of the cell, without causing too great a disturbance of the protoplasmic contents, and to harden materials of very soft texture. By this means we secure specimens showing the protoplasmic elements of the cell. The fixing agents in common use include formalin, picric acid, chromic acid, chromates, osmic acid and combinations of mercuric chloride with other salts. Formulæ for several fixing solutions will be found in the Appendix. Specimens should not exceed 8 mm. in size, else the fixing fluid will not rapidly penetrate the material. The time required for thorough fixing depends upon the texture of the material, the fixing fluid employed and the size of the specimen. After fixation the specimen must be thoroughly washed in water unless otherwise directed. If colored fixing fluids are used, it is well to continue washing until the wash water is free of color. If stained specimens are desired, the staining may be performed after washing

out the fixing fluid, although the advisability of staining at this point depends upon the type of stain employed and the kind of material one is working with.

Dehydration.—Paraffin or collodion may be used as a supporting medium in the infiltration process, but as both of these substances are immiscible with water, dehydration or removal of water from the cells is essential. The removal of water from cellular material must be accompanied gradually, and the water must be replaced by liquids miscible with the paraffin or collodion used as a supporting medium. Too rapid replacement of water would result in injury to the cell contents if not to the cell walls. As the process of dehydration is dependent upon the exosmosis of water and the endosmosis of the dehydrating fluid, the rapidity of the process is governed by the permeability of the cell walls. The dehydrating medium usually employed in vegetable histology is ethyl alcohol. After fixing and washing, the tissue fragments are placed in alcohol and water mixtures of gradually increasing concentrations, finally reaching absolute alcohol. The length of time the specimen should remain in each mixture depends upon the character of the tissues, woody structures requiring more time than those with soft membranous walls. In a specimen containing various kinds of tissue one must adjust the time of dehydration so as to secure full penetration of the thicker walled tissues. As a specimen is not likely to be injured by remaining too long in the alcohol and water mixtures, it is better to give the maximum time to each step in the process.

Dried materials may also be prepared for sectioning by the infiltration process, but in this case, fixing or

killing the tissues is unnecessary. The specimen is immediately immersed in the low-concentration alcohol and carried through in the manner prescribed for materials in which fixing is necessary. The details of the dehydration of materials may be summarized as follows:

1. Immerse specimen in alcohol 30 per cent for twenty-four hours
2.   "   "   "   50   "   "   "   "
3.   "   "   "   70   "   "   "   "
4.   "   "   "   95   "   "   "   "
5. "   "   "   "   absolute (a) for twenty-four hours
6.   "   "   "   "   (b) " six   "
7.   "   "   chloroform for three to six hours

Clearing.—The object of clearing is to make the cell walls and contents more translucent. Due to the action of the dehydrating medium, or on account of the nature of the specimen, the cell walls may be more or less opaque. The clearing agent corrects this condition and enables us to see structures which, because of this opacity, would not otherwise be apparent. In dealing with vegetable materials possessing thin and semi-transparent walls clearing may not be necessary. But even with such materials it is advisable to immerse the specimen, if only for a short time, in a clearing fluid in order to assure a more thorough penetration of the tissues by the paraffin. The use of chloroform after the absolute alcohol partly serves this purpose. The clearing agents employed include clove oil, cedar oil, turpentine oil, phenol and xylol and mixtures of these. After dehydration the specimens are placed in the clearing fluid for a period of from six to twenty-four hours, depending upon the texture and size of the specimen. The material should become almost transparent

in the clearing fluid. If the specimens become cloudy or white upon transference to the clearing agent, it is an indication that dehydration is incomplete. In this case the material must be returned to the stronger alcohol mixtures and a longer time allowed for dehydration.

Infiltration.—When the water within and around the cells has been replaced by chloroform, and the cell walls rendered transparent by means of a clearing medium, the next step is to introduce melted paraffin into the cavities of the cells and into the intercellular spaces. As previously stated, the paraffin serves as a supporting medium and prevents collapse of the specimen during section cutting. Melted paraffin will dialyse through cell walls, but the rate of dialysis can be materially hastened by immersing the specimen in a saturated solution of paraffin in chloroform or xylol. After this treatment the specimen may be transferred to melted paraffin kept at a temperature just above melting point. The paraffin for this purpose is especially prepared and the several grades obtainable range in melting point from 45° C. to 55° C. The grade to be employed depends upon the season and the texture of the specimen. In hot weather it is more satisfactory to use paraffin of higher melting point than that used in cold weather. The temperature of the paraffin bath must be kept fairly constant and slightly above the melting point. Infiltration will be slow or incomplete if the paraffin is too cold. If the temperature of the paraffin bath rises much above the melting point there is great danger of rupturing cell walls. The steps in the infiltration process are as follows:

1. Place the specimens in saturated solution of paraffin in chloroform or xylol for six to twelve hours.
2. Transfer specimens to melted paraffin (bath *A*) for two to four hours.
3. Transfer specimens to melted paraffin (bath *B*) for four to eight hours.

Embedding.—The last process in the infiltration method consists in surrounding the specimen with sufficient paraffin to support it during sectioning. Metal or paper molds are used for this purpose. The metal molds are adjustable for different sized specimens and permit more rapid chilling of the paraffin than is possible with paper molds. Melted paraffin is poured into the mold so as to form a layer about 8 mm. in depth. The mold is then placed in a shallow dish of cold water so that the paraffin on the bottom and sides will congeal quickly. The specimen is placed in the soft paraffin in the center of the mold, care being taken to place it so that the surface from which the sections are to be cut is toward the narrow end of the block and that the entire specimen is parallel with the long side of the mold. This *orientation*, or proper placing of the specimen, is necessary if one desires to obtain exact transverse sections and wishes to avoid frequent shifting of the block while cutting. After the specimen is properly placed, melted paraffin is poured into the mold so that the layers of paraffin on each side of the specimen are about equal in thickness. The filled mold is now rapidly cooled by immersion in cold water, care being taken to see that the water does not flow over the surface of the paraffin until a fairly thick pellicle is formed. Rapid cooling is necessary in order to prevent crystallization of the paraffin, as this would cause chipping

and crumbling during cutting. After the paraffin is thoroughly hardened the mold is removed, and the block containing the specimen is trimmed so that approximately equal thicknesses of paraffin surround the specimen on all sides. The blocks are most satisfactory when they are small; their length should not exceed 25 mm.

The placing of the specimen in the block depends upon the view or aspect of the object one desires. *Transverse* or *cross-sections* are cut parallel to a plane extending at right angles to the long axis of the specimen. *Radial* or *radial-longitudinal* sections are cut parallel to a plane extending through the long axis of the specimen and passing through the center of the object. *Tangential* sections are cut parallel to a plane extending through the long axis of the specimen but not passing through the center.

SECTION CUTTING

Free-hand Sections.—The simplest method of obtaining sections is by means of the section razor or hand microtome. This instrument is similar to a heavy razor except that it is flat on one side and concave on the other. Although with practice one may readily cut a small number of fairly good sections by this means, it is difficult to secure uniformity as regards thickness and size of sections. The block containing the specimen is held between the thumb and forefinger of the left hand. The forefinger serves as a support for the flat side of the razor and the thumb should be a safe distance below the level of the block so as to avoid accident. The razor, held in the right hand, is drawn obliquely across the block, making a

complete slice of supporting material and specimen. Moistening the block with a mixture of glycerin, alcohol and water is advisable, and the sections should be transferred to this liquid as soon as cut.

Microtome Sections.—The microtome is a mechanical device for cutting sections. It consists of a clamp to hold the block and knife for cutting the sections.
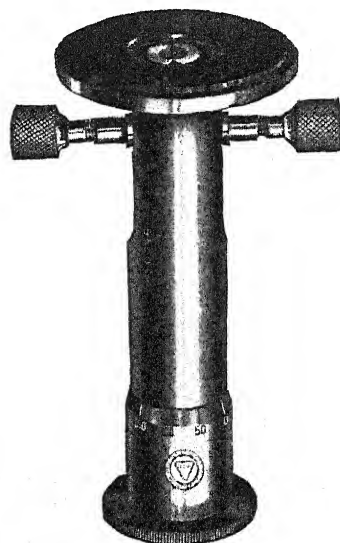
FIG. 1.—Hand Microtome.
(Bausch & Lomb.)

The object-clamp is so fitted that one may cut sections of definite thickness. In the simpler forms (Fig. 1), the hand microtome is used instead of a special blade, and the object-clamp is fitted in a screw-thread, so that the block may be moved to definite distances above the level surfaces upon which the flat side of the section razor rests. The milled head by which the object-clamp is raised upon turning through the screw-thread is usually graduated in micro-millimeters. The more complex forms of microtomes may be divided into sliding (Fig. 2), and rotary (Fig. 3) types. The sliding microtomes are further subdivided into those in which the object-clamp is fixed and the knife is movable (Fig. 2), and those in which the object-clamp is movable and the knife is fixed (Fig. 3). The type with sliding knife

is less liable to injure delicate specimens in cutting, because the block does not come into as sudden contact with the knife as in those machines in which the knife is rigid. Rotary microtomes are usually of the fixed-knife type and are so constructed that the object is fed toward and brought into contact with the knife at each revolution of a flywheel. Thickness of section is adjusted by means of a graduated cam, which limits the movement of a pawl in contact with a large toothed wheel, the shaft of which is threaded and inserted in the object-clamp. As the object strikes squarely against the knife edge, this type of microtome can only be used for sectioning fairly soft materials and those which are of even texture throughout. It is the most rapid in operation, as one need only turn the flywheel to obtain sections. All types of microtomes should be kept free of dust and all moving parts should be well greased or oiled. Microtome knives must be kept well sharpened and should show a straight edge without overhang when examined under low magnification. Different types of knives are required for objects of various textures, and each should be reserved for its particular purpose. In general, a heavy knife for wood and a second knife for materials of medium texture will answer all purposes and cover a fairly wide range. Knives having a flat side should be sharpened only on the concave surface, with perhaps a few finishing strokes on the flat side.

Microtome Technic.—The larger laboratory microtomes are provided with an object clamp in which the block may be placed, or the block may be attached to the object discs accompanying the appa-

ratus. Blocks may be attached to the corrugated side of the disc by warming the latter and firmly pressing the block against the rough surface. While these object discs are very convenient, they can only be used for small and even-textured materials. For large or hard-textured specimens the block must be secured in the object clamp, thin strips of cork being placed on the sides in contact with the gripping surfaces. These cork strips serve as buffers and allow
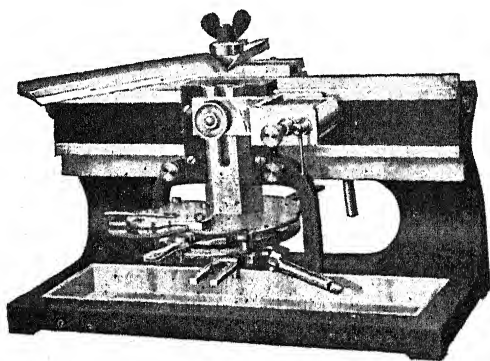
FIG. 2.—Automatic Laboratory Microtome.
(Bausch & Lomb.)

one to draw up well on the tightening screws with little danger of injury to the block. After the block is firmly fastened, place the knife in position so that it clears the block and object-clamp. The position of the knife varies with different embedding media. For objects embedded in paraffin the knife should be placed at an angle to the block. For objects in celloidin or collodion the knife should be placed straight, so that the block strikes it squarely. The knife should be adjusted so that the cutting edge is slightly

lower than the flat side, otherwise one will have a continuous resistance to the block at each stroke. After the block is secured and the knife properly placed, ascertain the thickness of section desired and set the indicator at the proper figure. Adjust the object-clamp so that the block is within a few millimeters of the knife edge and trim away the paraffin
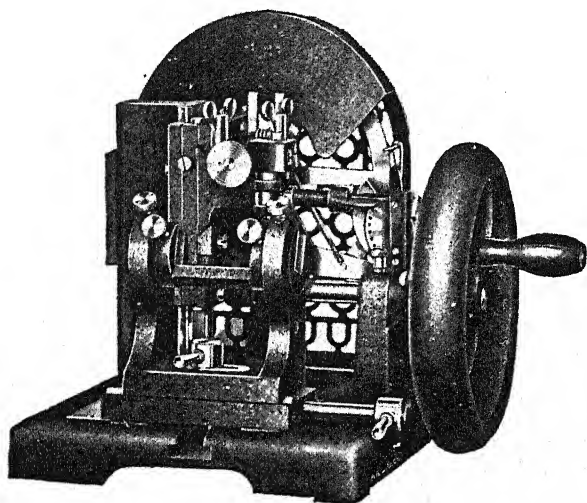
FIG. 3.—Minot Microtome.
(Bausch & Lomb.)

from the upper surface of the block until the specimen is visible. Move the object-carrier toward the knife with a slow and steady stroke, or if operating a rotary machine, turn the wheel steadily and not too rapidly. After cutting a few sections it is well to examine them under the microscope. If the sections are not cutting true or through the desired plane, use the adjusting screws located near the object-clamp

and make repeated adjustments until satisfactory
sections are secured. If the object is fairly straight
the adjustment need not be changed after true sections
are obtained. With bent or twisted objects con-
tinuous change of adjustment is necessary. Be sure
that the block is firmly clamped and tighten occa-
sionally.

Ribbon Sections.—At times it is desirable to cut
a series of sections showing the variations in structure
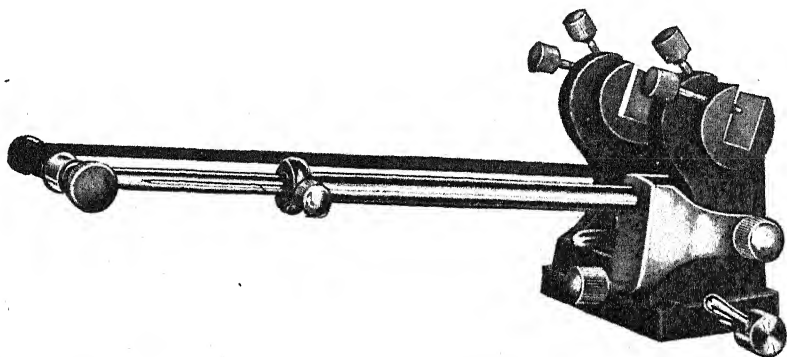
Fig. 4.—Ribbon Carrier.
(Bausch & Lomb.)

in different parts of a plant organ. This is accom-
plished by cutting in such a manner that each section
with its adherent paraffin forms part of a continuous
strip or ribbon in which each section follows the pre-
ceding in regular sequence. This operation is only
possible with small objects, as one cannot conveniently
manage or mount the long ribbons resulting from large
specimens. Success in ribbon sectioning depends upon
the texture of the paraffin, the shape of the block and
the temperature of the knife. Very soft paraffin

must be used and crystallization during embedding must be prevented. The edges of the block must be accurately trimmed so that the opposite sides are parallel, and the knife kept warm during the operation. Rotary machines give better ribbons than sliding microtomes. In cold weather very good results may be obtained by placing the machine on a radiator. After the first few sections have been cut, they should be gently drawn away from the knife and attached to a revolving drum (Fig. 4) so that the entire series may be kept intact. Curling of sections may be partially overcome by warming the block, as this fault is usually the result of using hard paraffin. Frictional electricity is occasionally a disturbing element, but this may be overcome by running a ground wire between the machine and a water or gas-pipe.

Removal of Embedding Medium.—Unless the sections are to be stained they are now immersed in xylol to dissolve the paraffin. It is best to use two xylol baths, draining the sections after the first immersion and using fresh solvent for the second. After being washed in xylol the sections may be mounted in balsam or may be brought into aqueous media by reversing the operations described in the section on Dehydration. If the sections are to be stained, and particularly if one is working with serial or ribbon sections, each section with the adhering paraffin is laid flat upon a clean slide previously prepared so that the sections will adhere. Slides for this purpose are coated with a mixture of egg albumin and glycerin (1 : 1) and then dried at a moderate temperature. Just previous to use, the albuminous film is moistened with water. The prepared slide with the specimens

properly arranged is placed upon a warm plate or in a low temperature oven and gently heated so that the water will evaporate. Upon evaporation of the water, the specimen will be found firmly fixed to the slide. Melting of the paraffin during this operation is of no consequence. After being thoroughly dried, the slide is immersed in xylol to dissolve the paraffin. As aqueous solutions of stains are usually employed, the specimen must be partially rehydrated by immersion in alcohol 95 per cent, 80 per cent and 70 per cent, at least three minutes being allowed in each concentration. The stain is now applied (refer to Chapter IV, Staining), after which the specimen is again dehydrated by passing through alcohol 70 per cent, 80 per cent, 95 per cent, absolute No. 1 and absolute No. 2, at least five minutes being allowed in each bath. After dehydration the specimens are cleared and mounted in Canada balsam.

EMBEDDING METHOD FOR NON-INFILTRATED SPECIMENS

Infiltration methods for the preparation of sections are not usually employed in the routine examination of foods and drugs, as fairly good sections may be secured by more direct methods. We may divide the materials likely to be encountered into (a) those received in fresh condition or of soft texture and (b) those received in dried condition or of hard texture. The details of preliminary treatment differ slightly in each instance.

Fresh Materials.—Many leaves, seeds and fruits which may be classed with this group require no preliminary treatment before blocking, as they are of

proper texture for direct sectioning. Other materials, including the fleshy parts of soft fruits, are too soft for sectioning and must receive a preliminary hardening treatment. Hardening may be accomplished by immersion of small pieces of the material in 6 per cent formalin solution or in 50 per cent alcohol. The length of time required for hardening varies according to the texture of the material. Prolonged hardening is to be avoided, as it tends to make the specimen brittle and is apt to cause distortion of the cellular elements.

Dried Materials.—Dried or hard materials should be softened before blocking. Softening may be accomplished by soaking the specimen in a mixture of glycerin, alcohol, and water (1 : 1 : 1), or by boiling in water. While the glycerin mixture is slower in action it has an advantage in that with it there is little danger of rendering the specimen too soft. Gentle heating will hasten the process. With materials of exceptional hardness boiling is necessary, and small amounts of acid or alkali may be added to aid in the softening.

Blocking for Cutting.—As most objects are too small to be conveniently held during sectioning, it is necessary to use a supporting medium. The specimen, if fairly soft, may be placed between slices of fresh potato or carrot and thus held firmly enough for sectioning. The objection to this method of blocking is that it is often difficult to separate small portions of the blocking material from the sections, especially if the latter are of small size. Pieces of elder pith may be used as a holding medium for materials of soft texture. Paraffin is the best holding medium for non-infiltrated specimens, as it may be

used with materials of almost any texture. Furthermore it can be readily separated from the sections, and by merely dipping the cut surfaces of the block in melted paraffin the specimen may be preserved for future reference. Paper or metal molds are prepared as directed in the section on Infiltration. The paraffin should have a melting point not higher than 60° C. A layer of paraffin is poured into the mold and cooled, the surface of the specimen is dried, after which it is dipped into the melted paraffin and placed in proper position upon the layer of paraffin already in the mold. Melted paraffin is now poured into the mold so as to cover the specimen, enough being added to form a substantial layer over the latter. It must be borne in mind that paraffin undergoes considerable contraction upon cooling. After the paraffin is thoroughly hardened the mold is removed and the block trimmed to convenient size. Sections may be cut by hand or by machine according to the methods outlined in the section on Infiltrated Materials. The removal of paraffin from the specimens is readily accomplished by placing the sections with the adhering paraffin in a beaker, adding water and gently heating. With most specimens a complete separation is thus effected, as, upon cooling, the paraffin accumulates on the surface of the water and the sections remain at the bottom.

SYNOPSIS OF SECTIONING METHODS

Infiltration Method:
  1. Fixing and hardening.
  2. Washing to remove fixing agent.
       (a) Staining may be desirable at this point.
  3. Dehydration.

4. Clearing.
5. Infiltration.
6. Embedding.
7. Sectioning.
   (a) Fixation of specimen on slide, if necessary.
8. Removal of infiltration medium.
   (a) Mounting in Canada balsam.
   (b) Complete rehydration if specimens in aqueous media are desired.
9. Partial rehydration.
10. Staining.
11. Removal of excess stain.
12. Dehydration.
13. Clearing.
14. Mounting in Canada balsam.

Non-infiltration Method:

1. Hardening of soft materials or softening of hard materials.
2. Blocking.
3. Sectioning.
4. Removal of blocking material.
   (a) Staining may be accomplished at this point.
5. Mounting in glycerin jelly.

POWDERING METHODS

Sections of plant parts are invaluable for showing the extent and relationships of the various tissues, but frequently the food and drug substances to be examined are in powdered form. One should, therefore, not only familiarize himself with the appearance of cellular elements and cell contents as they occur in the sectioned material, but should also be able to recognize these structures in powdered material even though they be partially disintegrated. The degree of disintegration varies with the comparative texture of the various tissues and the fineness of the powder. Hard tissues, such as fibrous and vascular elements, resist powdering better than epidermal and parenchymatic tissues of softer texture. Therefore, a

powdered sample will frequently show fibers and vessels in unbroken condition while the parenchyma cells are so disintegrated as to be difficult of recognition. For this reason better results will be had with powders sufficiently fine to separate the harder tissues but coarse enough to retain the cellular structure of the soft-textured elements. It is seldom desirable to work with powdered material finer than that which will pass through a 60-mesh sieve; and for materials that do not contain large amounts of woody tissues a 40-mesh powder is more advisable.

In general, there are four methods of reducing food and drug materials to powdered condition. Each of these has its limitations and one must be guided by experience and conditions in the selection of the method to be employed.

1. *Crushing.*—This method is useful in dealing with small amounts of friable substances, as starch and resins. The material is placed upon a slide and subjected to firm pressure with a spatula. Addition of a few drops of water or other mounting media may be advantageous in preventing the particles from being lost during the crushing.

2. *Filing.*—Sufficient powdered material for examination may often be obtained through the use of a fine file, especially if one is dealing with substances of hard texture and the amount of material is limited. Files used for this purpose must be carefully cleaned after use. This can be done by boiling in water and thoroughly drying in an oven. A new file should be cleaned before use, and rusty files should be discarded.

3. *Mortar Grinding.*—For ordinary laboratory work, this is the usual method, and the material is ground and sifted to the desired fineness. A porcelain mortar may be used in a majority of instances and has the distinct advantage of being readily cleaned. For extremely hard materials a small iron or bronze mortar is preferable, although difficult to clean after using.

4. *Milling.*—Where large amounts of material are to be reduced to powder so that a representative sample may be obtained, milling apparatus should be used. The kinds of milling apparatus range from the household coffee mill to regulation sample mills of the ball or plate type. Much can be accomplished with a small coffee mill if the material be well broken before milling. The larger power mills are more efficient, both in time and thoroughness, but are necessarily more expensive.

CHAPTER II

MOUNTING OF SPECIMENS

THE preparation of objects or specimens for observation by means of the microscope is termed *mounting*. This operation consists in placing the specimen upon a glass slide, surrounding it with a suitable mounting medium and covering it with a thin piece of glass known as a *cover-slip* or *cover-glass*. The slides employed are usually 25 by 75 millimeters in size and should be clear of flaws.

The purpose of the mounting medium is threefold; first, to correct excessive differences in refractive index between the specimen and the air; second, to fix the specimen in place; third, to preserve the specimen for future use. Comparatively few vegetable materials can be examined satisfactorily without the use of mounting media. To obtain clear views, contrast is necessary; and this can only be obtained through the use of mounting media of suitable refractive index. The refractive indices of specimen and mounting medium should be neither too near nor too far apart and the ideal mounting medium is between these extremes.

The cover-slip serves to fix the specimen in place, checks excessive evaporation of the mounting medium, prevents dust from lodging upon the specimen and protects the lenses of the microscope. It is essential

that slides and cover-slips be thoroughly cleaned before mounting specimens. These slips of glass are usually covered with a film of dirt which cannot be removed by simple polishing. They should be boiled in water containing a trace of ammonia, transferred to alcohol and dried with a silk or linen cloth. The cleaning mixtures listed in the appendix are very efficient. The use of cotton or woolen cloth for cleaning glassware or lenses is objectionable because of the fibers which adhere to the glass and which may be mistaken for parts of the specimen under examination. Clean slides and cover-slips should be kept free from dust and handled only by their edges. Finger prints which are almost invisible to the naked eye are very apparent when highly magnified.

MOUNTING MEDIA

Mounting media are classified as temporary and permanent. Temporary mounting media include fixed oils, volatile oils, water, glycerin, chloral hydrate solutions and alcohol. Mixtures of water and glycerin with or without the addition of alcohol may often be used to advantage. The oils and chloral hydrate solution may be used in mounting specimens containing so much fatty material that cloudiness would result from attempts to mix them with aqueous media. Temporary mounts are well adapted to routine examinations of food and drug samples, where preservation of the specimen is not essential and where the work upon a sample does not extend over too long a period of time. Owing to evaporation of the medium, if it be water or alcohol, or absorption of moisture if it be glycerin, these temporary mounts cannot be

preserved without special treatment. A further disadvantage of temporary mounts is that they cannot be readily transported.

The permanent mounting media include glycerin jelly, Canada balsam and solutions of various resins. Glycerin jelly is used in most instances because it is readily miscible with the aqueous or alcoholic media generally used for the preservation of stock specimens. Glycerin jelly is prepared with gelatin, glycerin and water with a trace of phenol as a preservative (refer to Appendix). Specimens mounted in glycerin jelly will last at least five years if kept under favorable conditions. Canada balsam is a liquid oleoresin obtained from the tree *Abies balsamea*. It is a thick liquid of pale yellow color and when used as a mounting medium is usually mixed with xylol. As it is immiscible with water it cannot be used unless the specimens are dehydrated by being passed through increasing strengths of alcohol and finally through xylol or chloroform. The balsam gradually solidifies and forms a varnish. The mounts are absolutely permanent, but crystals of resin acids may separate from the balsam and obscure the specimen. The refractive index of Canada balsam is less than that of glycerin jelly; therefore when working with very delicate objects mounted in balsam it is customary to stain or color the specimens so that clearer views may be had.

PREPARATION OF GLYCERIN JELLY MOUNTS

Materials required:
  Slides and cover-slips.
  Knife.
  Needles.

Forceps.
Glycerin jelly.
Alcohol lamp or Bunsen burner.

Operation:

1. Arrange the requisite number of clean slides in regular order and attach temporary labels, or number with ink.
2. Obtain specimen. These materials are usually preserved in a mixture of equal parts glycerin, alcohol and water.
3. Cut a cube of glycerin jelly (⅛ inch) and place it near the specimen.
4. Clean the needle by passing through the flame, removing the carbonized material by wiping upon clean paper.
5. Melt the cube of glycerin jelly by holding the slide a few inches above the flame. If mounting sections of plant parts, allow the melted jelly to flow over the specimen and proceed to step 6. If mounting powdered materials, make an intimate mixture of melted jelly and specimen.

    CAUTIONS.—Keep the slide warm, use the point of the needle for mixing; secure even mixture; keep mixture within small area; use as little heat as possible; remember that boiling causes formation of bubbles and spoils the specimen.
6. Pick up clean cover-slip with forceps and slightly warm it; allow it to touch the mixture of melted gelatin and specimen; withdraw the forceps, so that cover-slip gradually comes into contact with entire mixture. If excessive amounts of jelly have been used it will be necessary to press gently on the cover-slip, forcing out the excess, so that the mount will not be too thick for observation.

    CAUTIONS.—Do not track jelly on upper surface of cover-slip; do not attempt removal of excess jelly at this time.
7. Clean needle immediately, as directed in (4).

After mounting specimens allow them to remain flat until the jelly hardens. Excess jelly should not be removed until a few days after mounting, as this medium hardens slowly. To remove excess, scrape off as much as possible with a knife and then rub gently with silk or linen cloth moistened with alcohol.

Greater permanency may be had in glycerin jelly mounts by applying a varnish around the edges of the cover-slip. This process is termed *ringing*, and if carefully done effectively seals the specimen. Aside from cements and varnishes of secret composition, gold size, asphaltum and Venice turpentine may be used in ringing glycerin jelly mounts. Formulæ for the latter two varnishes will be found in the Appendix. In ringing specimens with round cover-slips, a simple turntable will be very convenient.

PREPARATION OF CANADA BALSAM MOUNTS

Materials required:
> Clean slides and cover-slips.
> Needles.
> Forceps.
> Tube of Canada balsam.
> Alcohol lamp or Bunsen flame.

Operation:
1. Place numbered or labeled slides in regular order.
2. Obtain specimen. This material must be thoroughly dry or suspended in xylol, chloroform or turpentine oil.
3. Place small drop of balsam upon specimen.
4. Clean needle.
5. Warm the balsam slightly and mix the specimen thoroughly with it.
6. Place cover-slip upon the mixture of balsam and specimen.
7. Clean the needle used for mixing.

Permit the mounted specimens to remain flat for a few days, after which time the excess balsam may be removed with xylol or turpentine.

It is essential that the simple operation of mounting specimens be performed with care and cleanliness. Defective mounts make future observations difficult, if not impossible. Small dust particles and finger prints are of consequence when highly magnified and may seriously interfere with good work.

CHAPTER III

THE MICROSCOPE

THE microscope is an optical instrument which produces an enlarged image of a near and usually small object. It differs from the telescope in that the latter instrument produces enlarged images of objects so far distant from the observer that they appear small although they may be very large. The human eye cannot distinguish objects below a certain size, because the extreme light rays proceeding from such an object are so close that the image cast upon the terminations of the optic nerve or *retina* is too small to cause the stimulation necessary for vision. The function of the microscope lenses is to increase the distance between the extreme rays cast by a small object, and thus render the image received by the retina large enough to cause sufficient stimulation for vision. Magnification by a lens depends upon its ability to refract light rays. This property is in turn dependent upon the principle that light rays passing through objects of differing densities are changed in direction. Light rays passing from a medium of lesser to one of greater density will be bent toward a line at right angles to the surface of the denser medium (Fig. 5). Light rays passing from a medium of greater to one of lesser density will be bent away from a line perpendicular to the surface of the medium of lesser

density (Fig. 5). Glass being of greater density than air, light rays passing through it will be bent toward a line at right angles to its surface.

Six different types of lenses are formed by combinations of curved surfaces, either with each other or with flat or plane faces. A lens similar to an ordi-
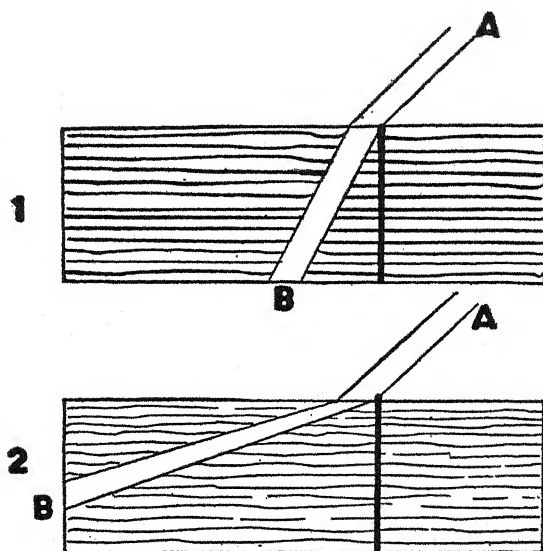
FIG. 5.—Refraction of Light Rays.
1. Path of ray passing from less dense medium (*A*) to one of greater density (*B*).
2. Path of ray passing from medium of greater density (*A*) to one less dense (*B*).

nary magnifying glass is termed *convex* or *double convex*. If the lens is thinnest at the center it is termed *concave* or *double concave*. Two variations of convex lenses are recognized, *plano-convex* and *concavo-convex* or convergent meniscus. Similarly there are two variations of concave lenses, *plano-concave* and *convexo-concave* or divergent meniscus. The six types of

lenses are illustrated in Fig. 6. By reference to Fig. 7, it will be seen that light rays passing through the central part of a lens are not refracted. A line passing through the thickest part of a convex lens and the thinnest part of a concave lens is termed the *optic axis* or *principal axis*. Light rays passing through a convex lens are refracted toward the optic axis, whereas in concave lenses they are refracted away from the optic axis (Fig. 7). The extent of refraction or bending of light rays differs in different parts
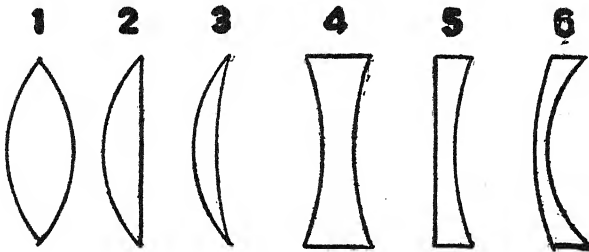
Fig. 6.—Types of Lenses.

1. Double convex. 2. Plano-convex. 3. Convexo-convex. 4. Double concave. 5. Plano-concave. 6. Concavo-concave.

of the lens. Rays entering the edges of a lens are refracted to a greater extent than those entering nearer the optic axis; therefore light rays entering a biconcave lens are bent so that they meet at a point along the optic axis. The point at which these rays cross the optic axis is termed the *principal focus*. The distance between lens and principal focus is termed the *focal distance,* or *focal length,* of the lens. A biconvex lens held in the sun will illustrate the foregoing, as it will so concentrate the light rays at a certain point that they will slowly ignite materials placed at the principal focus.

For purposes of magnification objects are placed either beyond or within the focal point. While in both instances magnification is apparent to the observer, it will be found that the reasons for these apparent enlargements of the image are different in
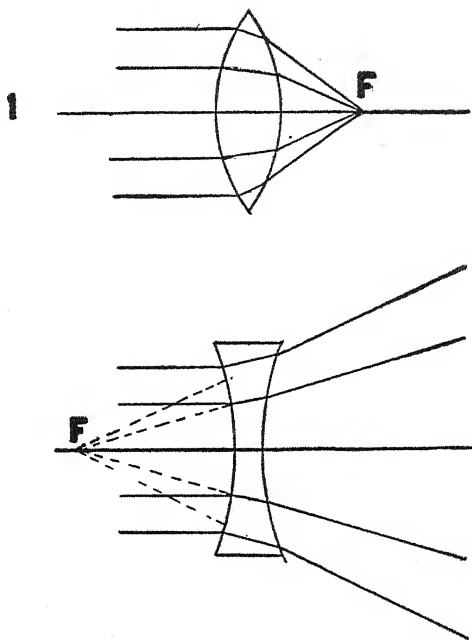
FIG. 7.—Refraction of Light Rays by Lenses.

1. Double convex lens causes convergence of rays to focal point (*F*).   2. Double concave lens causes divergence of rays from focal point (*F*).

the two cases. Objects placed beyond the focal point of a lens will give rise to enlarged images, termed *real images*, which can be projected upon a screen. The degree of enlargement is directly proportional to the distance of the screen from the lens. Objects placed at a point within the focal distance of a lens

will also give rise to enlarged images; but these cannot be projected upon a screen and are termed *virtual images*. The changes in direction which light rays undergo in the production of a real image are entirely due to the refractive effects of the lens (Fig. 8). The apparent enlargement in the production of a virtual image is due to the action of the lens supplemented by the tendency of the eye to follow light
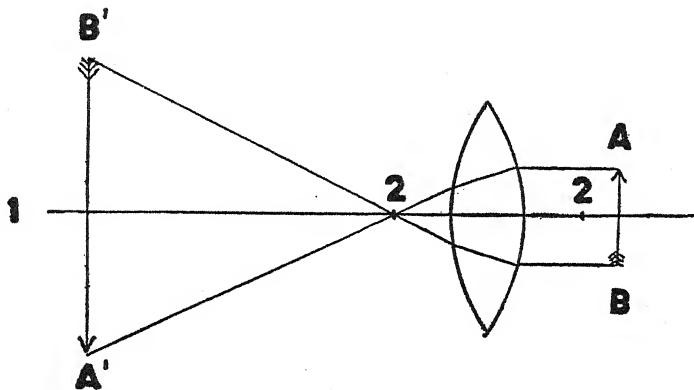
Fig. 8.—Formation of Real Image by Convex Lens.
1. Optic axis.   2. Focal points.   *A–B*. Object beyond focal point (2).   *A′–B′*. Real Image of object *A–B*.

rays through a lens disregarding the refractive effects of the latter (Fig. 9).   Real images always appear inverted whereas virtual images appear erect.

Spherical and chromatic aberration are properties of lenses which formerly caused considerable difficulty. *Spherical aberration* is due to the fact that light rays entering the edges of a lens undergo greater refraction than those entering nearer the optic axis (Fig. 10). These differences in the amount of refraction cause the rays entering the center of the lens to come to a focus

at a greater distance from the lens than those entering the edges; and the resulting differences in focal points
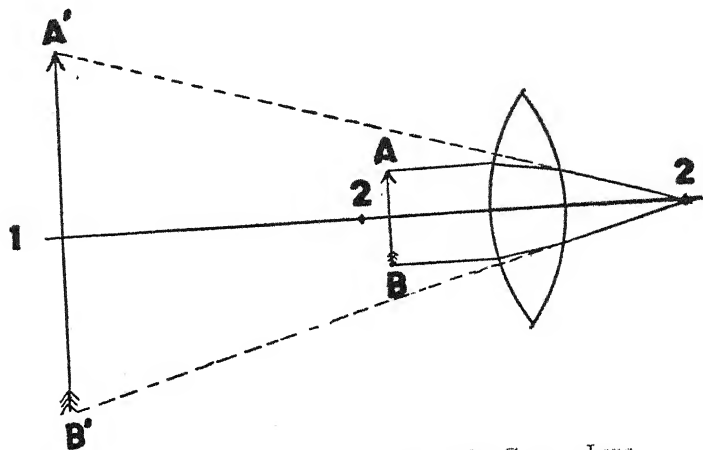
FIG. 9.—Formation of Virtual Image by Convex Lens.

1. Optic axis. 2. Focal points. *A–B*. Object within focal point (2). *A'–B'*. Virtual image of object *A–B*.
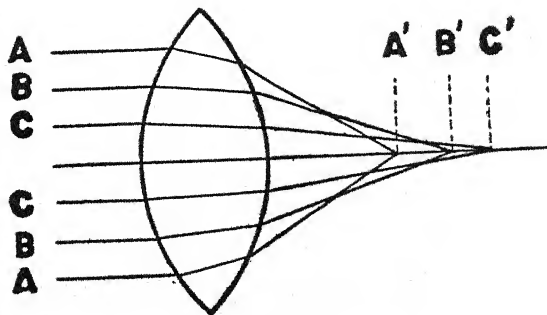
FIG. 10.—Spherical Aberration.

A–B–C. Light rays passing through different parts of the lens are brought to different foci, *A'*, *B'*, *C'*.

give rise to blurred images. Ordinary or white light is composed of rays of different lengths, each of which

produces a specific color. A glass prism, of which
a lens is but a modification, has the power of separat-
ing white light into its component rays which are
of different lengths, and consequently of different
colors. The play of colors resulting from the passage
of light through a prism or lens is termed the spectrum
and will show the colors in the following order: red,
orange, yellow, green, blue and violet. The red rays
are the longest and the violet rays are the shortest
(Fig. 11). Owing to the difference in wave lengths
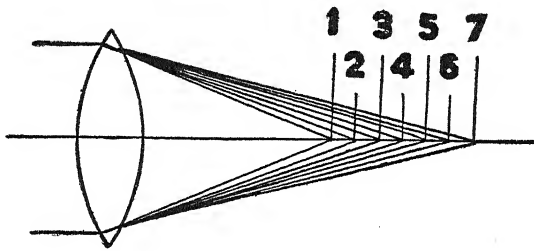
FIG. 11.—Chromatic Aberration.

The lens acts as a prism and causes dispersion of white light into rays of different colors. 1. Ultraviolet focal point. 2. Violet focal point. 3. Blue focal point. 4. Green focal point. 5. Yellow focal point. 6. Orange focal point. 7. Red focal point.

the red rays will come to a focus at a greater distance
from the lens than the violet rays. These differences
in focal points give rise to blurred and colored edges
around the image and are responsible for the defect
termed *chromatic aberration*. Both of these defects
are corrected by using glass of different kinds in the
construction of compound lenses and combining dif-
ferent types of lenses. *Achromatic lenses* are those
in which chromatic aberration has been corrected.
Aplanatic lenses are those which have been corrected
for spherical aberration.

THE SIMPLE MICROSCOPE

The simple microscope or magnifying glass consists of a double convex lens or a combination of such lenses. The image produced appears erect, or right side up, and is a virtual magnification. The object is placed within the focal point of the lens (Fig. 9). While the field or area which can be magnified by a simple microscope is nearly as large as the diameter of the lens, the outer edges will show spherical and chromatic aberration unless the construction is such as to correct these faults. The magnification is comparatively low and seldom exceeds 25 diameters. The forms of simple microscopes range from the jeweler's magnifying glass or loup (Fig. 12), to complex instruments fitted with achromatic and aplanatic lenses and various
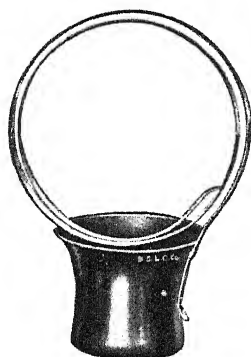
Fig. 12.—Jeweler's Magnifier or Simple Microscope.
(Bausch & Lomb.)
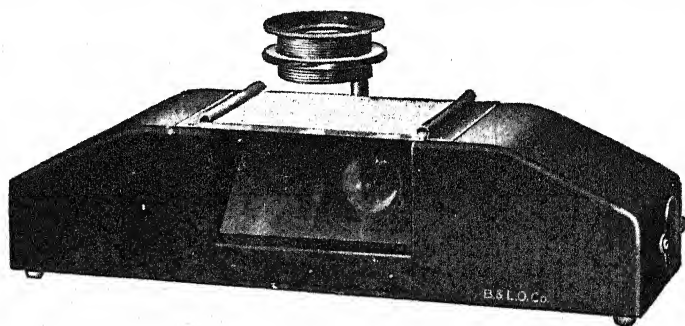
Fig. 13.—Simple Dissecting Microscope.
(Bausch & Lomb.)

mechanical accessories for greater convenience and accuracy in manipulation (Figs. 13 and 14). While the simple microscopes do not give high magnifications, they are of great service in work requiring views of an extensive field, as in engraving, watch
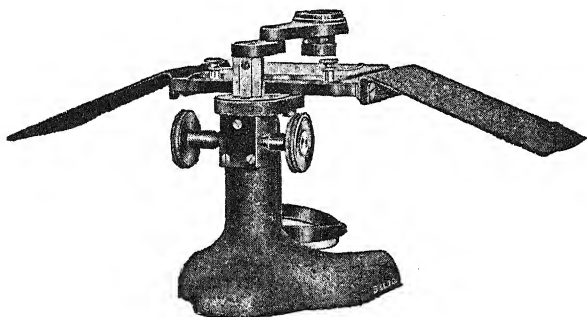
FIG. 14.—Dissecting Microscope.
(Bausch & Lomb.)

repairing, textile examinations and the examination of floral parts.

THE COMPOUND MICROSCOPE

The compound microscope consists of a series of plano-convex or converging lenses. These are mutually arranged so as to form two systems of lenses—an objective or lower combination and an ocular or upper combination. The objective, or set of lenses nearest the object, produces an enlarged image which is again magnified by the ocular lenses. Therefore the image produced by a compound microscope is the result of a double magnification. The object is placed beyond the focal point of the objective and the picture projected by this system of lenses is an inverted real

image. This image is formed at a point within the focal distance of the ocular; therefore the picture formed by the ocular will be a virtual magnification
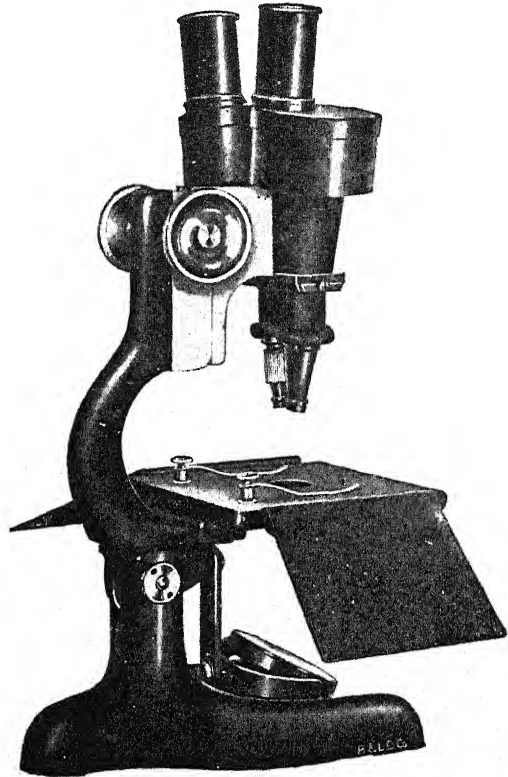
Fig. 15.—Binocular Microscope (Greenough Type).
(Bausch & Lomb.)

of the real image produced by the objective. The object as viewed through the compound microscope always appears inverted (Fig. 18), unless an erecting prism or other device is used to reverse the real image projected by the objective. The field or area which

can be viewed is always smaller than that covered
by the lens of a simple microscope. The magnifi-
cations obtainable range from 15 to 2500 diameters,
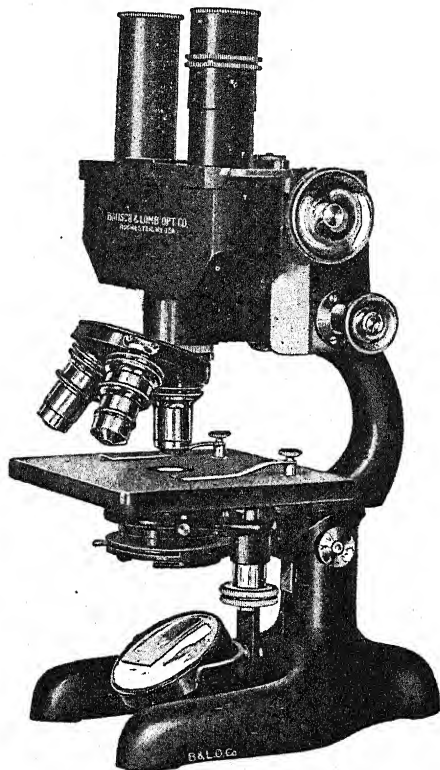
Fig. 16.—Binocular Monobjective Microscope.
(Bausch & Lomb.)

although little practical application is found for
magnifications above 1500 diameters. While the
great majority of compound microscopes in use are
of the monocular type, considerable progress has
been made in the production of the binocular forms.

The advantages of the binocular instrument are clearer images, due to the stereoscopic vision which is possible, and less fatigue upon use over a long period of observation. Both of these claims are well founded;
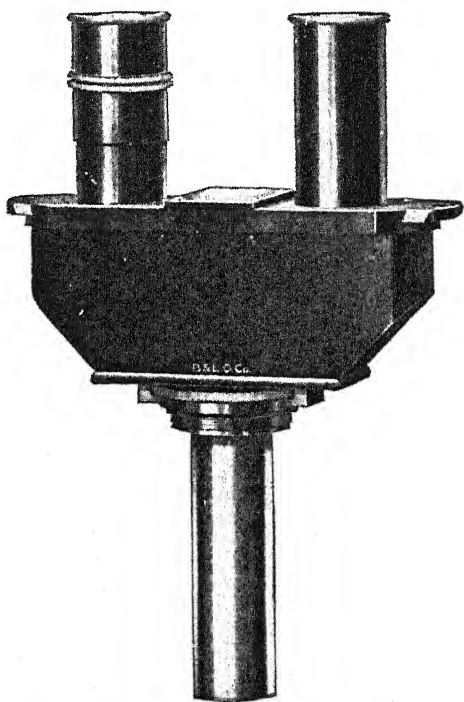
FIG. 17.—Binocular Eyepiece.
(Bausch & Lomb.)

but the disadvantages are greatly increased cost, the necessity of using paired objectives and oculars which cannot be transferred to any other instrument, and the inconvenience of not having one eye free for drawing work. The simpler forms of binocular microscopes are in reality two microscopes so mounted
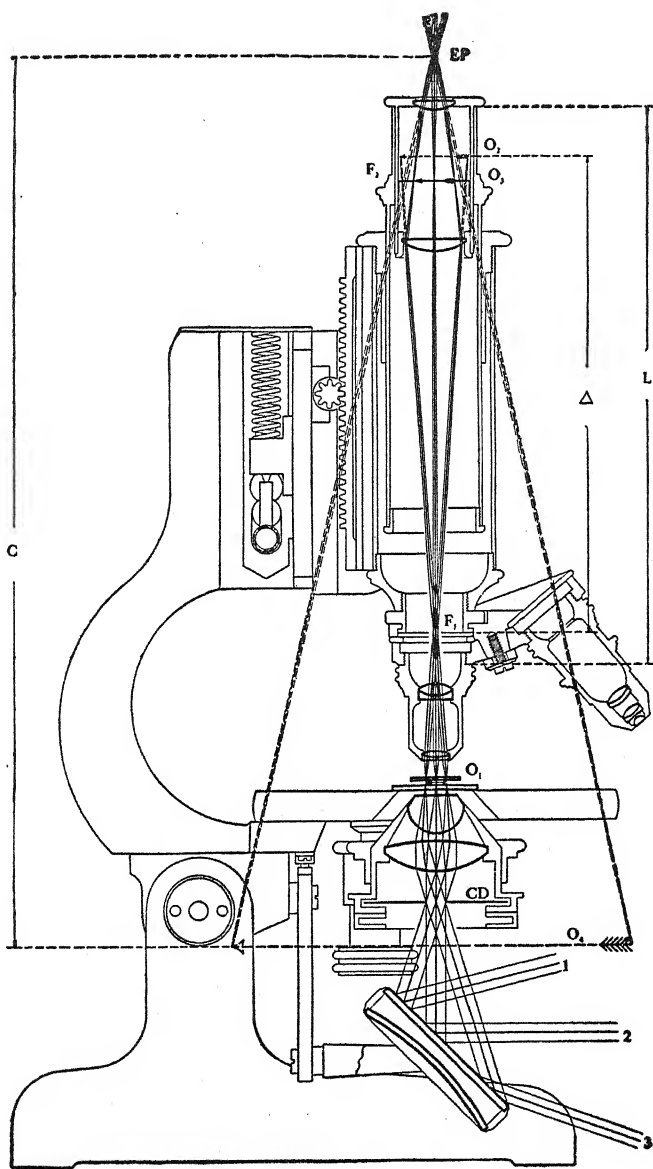
FIG. 18.—Diagram Showing Path of Light Rays.

F_1, Upper focal plane of objective. F_2, Lower focal plane of eyepiece. Δ, Optical tube length = distance between F_1 and F_2. O_1, Object. O_2, Real image in F_2 transposed by the collective lens, to O_3, Real image in eyepiece diaphragm. O_4, Virtual image formed at the projection distance C, 250 mm. from EP, Eyepoint. CD, Condenser diaphragm. L, Mechanical tube length (160 mm.). 1, 2, 3, Three pencils of parallel light coming from different points of a distant illuminant, for instance, a white cloud, which illuminate three different points of the object.

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as to focus upon a given area (Fig. 15), and in these
only comparatively low magnifications can be
secured. In the more improved types, single ob-
jectives of the usual powers are used and the body
tube is fitted with prisms which reflect the image
into parallel ocular tubes (Fig. 16). More recent
is the introduction of a binocular eyepiece (Fig. 17),
which may be fitted to the ordinary monocular,
monobjective microscope, and which has proven
very satisfactory. The principal parts of a com-
pound microscope are the objective and the ocular;
but mechanical devices are necessary to hold these
lens systems in proper relation to each other and to
the specimen. Proper support and illumination of
the object must also be provided for. It is therefore
customary, in considering the construction of a com-
pound microscope, to speak of the optical parts and
the mechanical parts.

OPTICAL PARTS OF THE COMPOUND MICROSCOPE

The optical parts of a compound microscope
include the objectives and oculars, together with the
condensing lens and the mirror.

Obejctives.—Microscope objectives consist of one
or more combinations of convex and plano-convex
lenses. The lower-power objectives consist of one
or two such combinations, while those used for higher
magnifications consist of three or four combinations
(Fig. 19). The degree of magnification produced by
different objectives is usually indicated by certain
markings upon the mounting. Various systems are
employed in the marking of objectives and oculars
to indicate degrees of magnification. Among these,

attention may be given the Continental, the English, the metric, and a recent practice of indicating the power of an objective directly in terms of diameters of magnification. The latter system is a distinct advance, in that the magnification of a given combination of objective and ocular may be readily computed without reference to tables. The Continental system of marking objectives is entirely empiral and consists of a series of numerals beginning with No. 1, the lowest power, and continuing up to No. 9, which
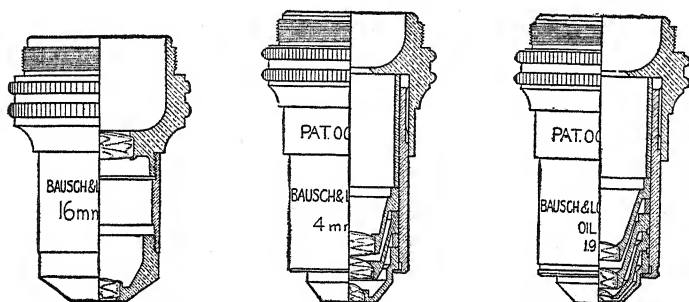
FIG. 19.—Sectional Views of Objectives.
(Bausch & Lomb.)

is the highest of the series. In the English system the relative powers are indicated in inches or fractions of an inch, usually beginning with the 2-inch objective as the lowest, and extending to the $\frac{1}{16}$ inch or highest power. This system is based upon what is termed the equivalent focal length of the lens. An objective marked $\frac{1}{6}$ would produce a real image of the same size as a simple convex lens, the principal focus of which is $\frac{1}{6}$ inch distant from the lens. The metric system of marking objectives is similar to the English system and differs from the latter only in that

magnification is expressed in millimeters of equivalent focal length, and not in inches or fractions of an inch. The objectives, according to this system, usually range from 48 mm., the lowest, to 1.5 mm., the highest. Briefly stated, the relation of marking to magnification is as follows: Continental system; lower numerals indicate lower powers; English and metric systems; lower numerals indicate higher powers. In the case of Continental objectives there is an exception to these rules. As previously stated, the highest powered Continental objective is ordinarily No. 9; but objectives above this power are manufactured, and are marked in fractions of an inch. Thus the sequence would be No. 9, $\frac{1}{16}$ inch, $\frac{1}{12}$ inch and $\frac{1}{15}$ inch. One may judge the power of an objective by the size of the end lens, as the lower power objectives have larger end lenses than those of higher power.

In lower power lenses the refraction of light rays as they emerge from the upper surface of the glass slide is not of great consequence. In working with lenses giving high magnifications (1.9 mm., $\frac{1}{12}$ inch, etc.), these refractions offer serious difficulty as great amounts of light are lost because of the small diameter of the end lens. This difficulty is overcome by placing liquids between the end lens of the objective and the slide. Air has a refractive index of 1.00, while the refractive index of glass is approximately 1.55. By placing a layer of water, which has a refractive index of 1.30, between the objective and the slide, we minimize these differences in refractive index, thus decreasing the number of light rays lost through refraction. Lenses that cannot be used without the interposition of liquids to prevent excessive loss of

light by refraction are termed *immersion objectives,* and the liquids used for this purpose are known as *immersion liquids.* Thickened cedar oil, of refractive index 1.55, is usually used as an immersion medium in bacteriological work (Fig. 20). Immersion lenses, while of importance in bacteriological and pathological investigations, are seldom used in vegetable histology. As immersion lenses are very expensive
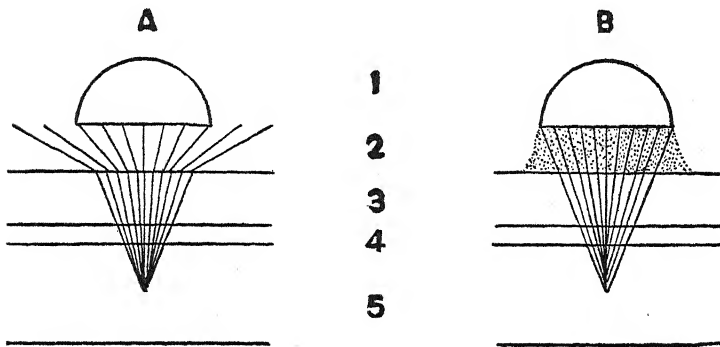
FIG. 20.—Immersion Objectives.

A. Unless immersion fluids are used, considerable light is lost through refraction. *B.* Very little light is lost through refraction if immersion fluids are used. 1. End lens of objective. 2. Space between objective and coverslip. 3. Coverslip. 4. Space occupied by specimen. 5. Object slide.

and easily scratched, they must be handled with care, and immersion fluids must be removed after use. This is best done by using soft lens paper moistened with xylol.

The *working distance* of an objective is the space which intervenes between the specimen and the end lens of the objective when the latter is properly focused upon the object. The working distance is in inverse ratio to the magnification, lower-power objectives having a greater working distance than those of higher

power. Many objectives bear figures indicating their power to distinctly show cellular structures separated from each other by very small distances. This property is termed *resolving power* and is expressed in terms of *numerical aperture* (N. A.). The resolving power of a lens is in direct ratio to its numerical aperture; therefore an objective of N. A. 1.30 will be of greater use in the examination of very fine details than an objective of N. A. 0.85.

Oculars.—As previously stated, the function of an ocular is to further magnify the magnified image produced by the objective. The usual type of ocular (Huyghenian or negative), consists of two plano-convex lenses separated from each other by considerable distance. A diaphragm or metal plate with a circular opening is placed between the lenses of the ocular. The magnification of an ocular is indicated by markings on the top plate or on the tube. In the Continental system, magnification of oculars is empirically indicated by numerals ranging from No. 1, the lowest, to No. 6, the highest. In the English system, degree of magnification is indicated in inches or fractions of an inch ranging, from 3 inch, the lowest, to $\frac{1}{2}$ inch, the highest. These figures have a fixed relation to the equivalent focal distance as explained under Objectives. The simplest method of marking oculars is by indicating the number of diameters (or times) they magnify the image formed by the objective. In this system the ordinary range of powers is from $5\times$ to $20\times$. Where high magnification with long working distance is desired, high-power oculars may often be used to advantage. The better types of oculars are corrected for spherical and chromatic

aberration. The magnifications obtainable with different combinations of objectives and oculars are stated in tabular form in the Appendix.

Condensing Lenses and Mirror.—The purpose of the mirror is to reflect light upon the object, thus illuminating it upon all sides. Illumination can be secured without use of the mirror; but upon viewing the object it will be found that one side is light and the other is dark. That portion of the specimen nearest the source of light is clearly defined, while that further away is obscured. Furthermore, in most instances we desire views of cell structure which can only be obtained by light passing through the object. One surface of the mirror is plane and the other is concave. The plane surface reflects parallel rays and the concave surface reflects converging rays, or concentrates the light. The plane surface is usually used where the source of light is near, and with instruments equipped with condensing lenses. The concave surface is used when the source of light is distant. It is also used when interfering objects, such as window frames or nearby buildings, partially cut off the light or are projected into view. When possible, the plane surface should be used when working with instruments equipped with condensing lenses.

The function of the condensing lenses is to concentrate the light, or bring the light rays to a focus upon the object, thus increasing the illumination of the latter. The *Abbé condenser* (named after its originator) consists of two or more modified planoconvex lenses (Fig. 21), and is attached to the microscope so that the top lens projects through the stage. The condenser mounting in modern instruments is

equipped with a focusing device which permits changes in the position of the focal point of the light rays passing through the condenser. In fine work, and with high powers, focusing of the condenser is essential. The usual types of Abbé condensers are not corrected for spherical and chromatic aberration. As the function of the condenser is merely concentration of light and not the production of images, these non-corrected lenses serve all ordinary purposes. In using immersion
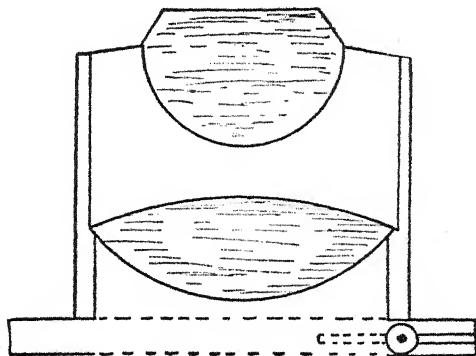
Fig. 21.—Abbé Condenser (two lens).

lenses one should place a layer of immersion fluid between the upper lens of the condenser and the lower surface of the slide; otherwise much light will be lost by refraction, in passing from the condenser to the object.

The *iris diaphragm* is usually included in the condenser mounting. Although this is a mechanical device, it is so intimately connected with the working of the optical parts that it should be considered in conjunction with these. Ordinarily an excessive amount of light is projected upon the object by the

mirror, and the image appears blurred. The iris diaphragm, by regulating the amount of light reaching the object, reduces this excessive illumination. While proper adjustment of the diaphragm opening is an important factor in securing clear-cut views, no absolute rule can be given for this adjustment. The size of the diaphragm opening varies with the strength of the light, the different combinations of objectives and oculars, the density of the object and the variations in the eye of the observer. Aside from the general rule that one must use larger diaphragm openings with higher power objectives, the proper adjustment of the diaphragm under different conditions must be learned by experience.

MECHANICAL PARTS OF THE COMPOUND MICROSCOPE

While advances in histology, bacteriology and the allied sciences have been coincident with the perfection of the optical parts of the compound microscope, improvements in the mechanical parts of the instrument have played an important part. High-power lenses demand extreme accuracy in focusing and firm, yet readily adjustable, support; therefore the modern microscope represents optical and mechanical ingenuity of the highest order.

The microscope rests upon a horse-shoe shaped casting termed the *base*. This base, of cast iron or brass, may be hollowed out, and the cavity filled with lead, to insure greater stability. Arising from the base is the *pillar*, which is usually jointed so that the instrument may be inclined toward the worker. At a point above the *inclination joint*, the *stage* is attached. The stage serves as a rigid support for the specimen,
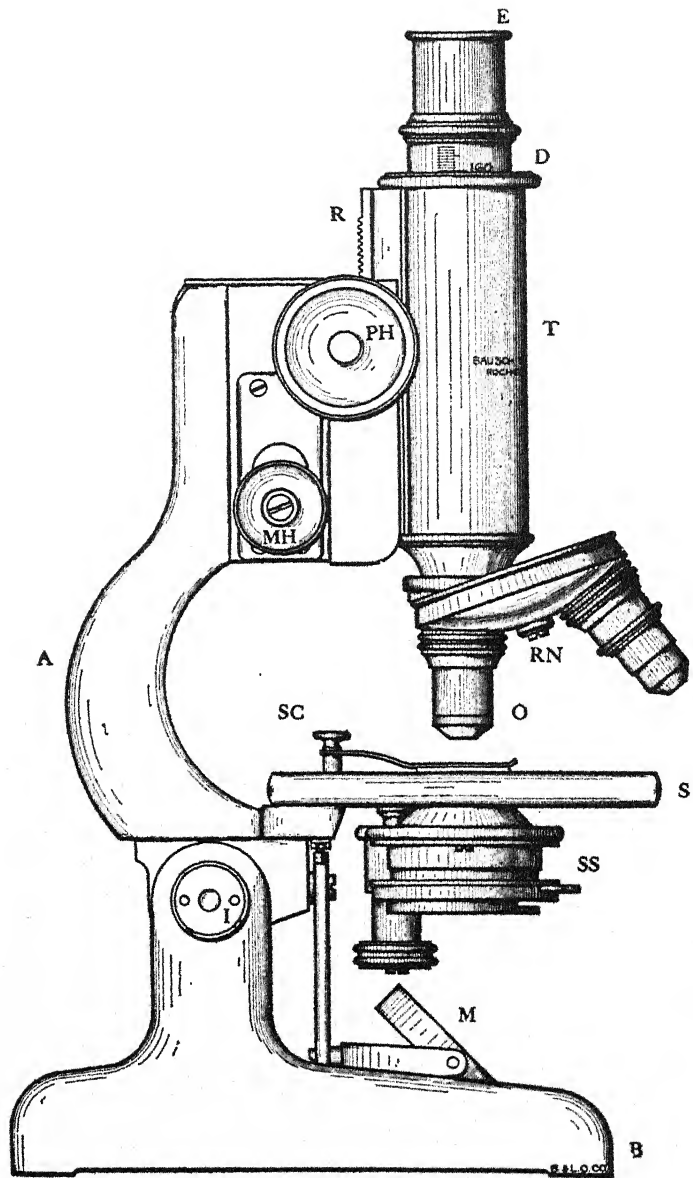
Fig. 22.—Parts of the Compound Microscope.

(Bausch & Lomb.)

E, Eyepiece; *D*, Draw Tube; *T*, Body Tube; *RN*, Revolving Nosepiece; *O*,
Objective; *PH*, Pinion Head; *MH*, Micrometer Head; *HA*, Handle Arm; *S*, Stage;
SS, Substage; *M*, Mirror; *B*, Base; *R*, Rack; *P*, Pillar; *I*, Inclination Joint.

and is perforated to accommodate the condenser. Branching from the upper part of the pillar is the *arm*, to which is attached the *tube* bearing the ocular and objectives. The tube is usually fitted with an inner tube or *draw-tube*, which may be used to vary the distance between objectives and ocular. The draw tube is usually graduated in millimeters and should be drawn out to the figure 160 (or 16) as this is the tube length for which microscope lenses are ordinarily corrected. The ocular slips into the upper end of the tube; but the lower end is provided with a circular metal plate, the *nosepiece*, with threaded collars in which the objectives fit. Nosepieces accommodate two, three or four objectives, and turn on a pinion so that the different objectives may be brought into place at the lower end of the tube. The condenser mounting, with iris diaphragm and mirror support, is usually attached to the under surface of the stage, and these devices are collectively known as the *substage*.

A microscope is fitted with two adjustments for focusing or changing the position of the tube, with relation to the specimen. The *coarse adjustment* consists of two large milled wheels moving a toothed pinion which is in mesh with a rack fixed on the tube. The adjustment is used with lower powers to secure an approximate focus. The *fine adjustment* is controlled by a milled wheel placed on top of the pillar, or by small wheels one on each side of the arm. The mechanism of the fine adjustment varies in different instruments, but in all cases it is the most delicate of the mechanical parts and should be handled with due care. Turning this adjustment toward the right, or

toward the microscope, lowers the tube, while turning
in the reverse direction raises it. The fine adjust-
ment is used with high-power objectives and for exact
focus with the lower powers. With high power object-
ives the lens is very near the slide and care must be
exercised in focusing with the fine adjustment, so that
the objective is not forced down on the specimen.
Modern fine adjustments are so constructed that when
the objective comes in contact with the slide, down-
ward motion ceases. The range of tube movement
with the fine adjustment is limited, and the lower
limit will be indicated by stoppage of the adjustment
wheels or an automatic reversal of tube movement.
Fine adjustments should not be turned more than
five revolutions in either direction; and one should
particularly observe this caution when working with
microscopes having the fine adjustment on top of the
pillar.

USE OF THE MICROSCOPE

To obtain good results with a microscope one must
be familiar with the construction of the instrument
and must pay attention to certain apparently insig-
nificant details. A regular order of procedure should
be observed and should be so well in mind that it
becomes more or less automatic. In this way the
worker is free to devote greater attention to the
examination of the specimen and less to the mechan-
ical details of focusing and lighting.

Position of Microscope and Worker.—The work-
ing tables in a laboratory should be so arranged that
the students can secure light from windows facing
north. North light is preferred because it is less

subject to variation. The instrument should be placed opposite the left shoulder and not more than four inches from the edge of the table. This permits one to use the right hand for drawing and the left for focusing or other adjustments. If one is left handed, the microscope should be placed opposite the right shoulder. Shifting the microscope further than a few inches from the edge of the table not only cuts off the light from the other students in the row, but necessitates an uncomfortable working position. Although it may be necessary to incline the instrument, better results will usually be obtained by keeping it upright. If working with temporary mounts, one must keep the microscope upright to avoid loss of the specimen. In any case the inclination should not be more than 45 degrees. If the student finds that when he sits upright his eye is below the level of the ocular, he should incline the instrument. When a microscope is carried, it should be kept upright, two or three fingers grasping the pillar below the stage. Otherwise the fine adjustment may be injured or the ocular lost.

Lighting.—After placing the microscope in working position and locating the different working parts, clean the lenses with cloth (soft silk or linen) or lens paper, turn the iris diaphragm to full opening and place low power objective (No. 3 or 16 mm.) in position, 5 mm. above the stage. Turn the mirror so that it half faces the north windows and, *while looking into the ocular*, move the mirror slightly in different directions until you obtain a clear white field. After securing proper illumination, do not move the microscope or the mirror. It is better to use the plane

mirror unless the light is poor. Direct sunlight should
not be used for illumination, as it is so strong that
it will injure the eyes of the worker.

Focusing with Low Powers.—After having obtained
proper illumination, place the slide, cover-slip side
upward, upon the stage so that the object is in the
center of the condensing lens. Lower the tube with
coarse adjustment so that the objective is about 3 mm.
above the specimen. *Never focus downward with the
coarse adjustment unless you are watching the distance*

Illuminating object with plane   Illuminating object with concave
mirror   mirror.
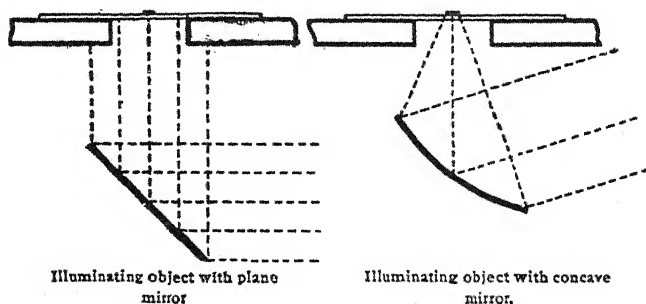
FIG. 23.—Illumination of Object by Mirror, without Condenser.
(Bausch & Lomb.)

between the objective and the specimen. Slowly focus
upward with coarse adjustment until the object is
visible. The distance between the end lens of the
low power objective and the specimen, when the latter
is in focus, should be between 6 and 10 mm. If you
find that you have raised the tube more than 10 mm.
above the specimen, you have probably missed the
focus and should repeat the operation, taking care
to focus upward *slowly*. The specimen will usually
be excessively lighted, so one must decrease the dia-

phragm opening until the proper degree of illumination is secured. Although the specimen is visible and well lighted, the finer details of structure may not be apparent and the fine adjustment should be used to obtain a clearer view. Turn the fine adjustment in each direction not more than five revolutions until best results are obtained. It will usually be necessary to change the fine focus for different parts of the specimen and the student should keep one hand moving this adjustment. As much time is
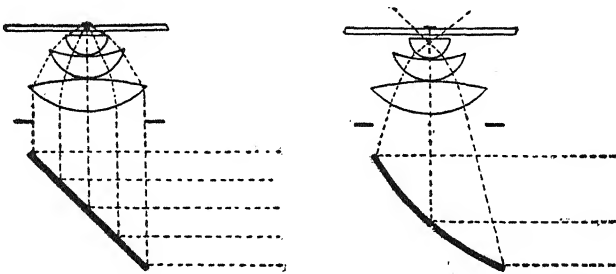
A. With plane mirror (correct way).   B. With concave mirror (incorrect way).

Fig. 24.—Illumination of Object by Mirror, with Condenser.
(Bausch & Lomb.)

saved by placing the object in the center of the low power field before changing to higher powers, specimens should always be located and viewed under low powers even if the high powers are subsequently used.

Focusing with High Powers.—Raise the tube by means of the coarse adjustment so that the objective is about 10 mm. above the specimen, and place the high power objective (No. 7, No. 8, or 3 mm.) in position. In the best instruments, the objectives are *par-focal*, or are so constructed that when one has focused the low-power objective, the other objectives

may be immediately turned into position and will be in approximate focus. Adjustments must be very accurate on par-focal objectives and the lenses can only be used on the instrument to which they have been fitted by the maker. As the lenses of high power objectives are smaller than those of low power, and admit less light, the diaphragm opening should be slightly increased. Lower the objective, using the coarse adjustment, until it almost touches the cover-slip, then slowly focus upward until the object is visible. A final regulation of the diaphragm is usually necessary; and the student should keep focusing with the fine adjustment so as to obtain good views of all parts of the specimen. As previously stated, the lens systems of microscopes are corrected for use with a certain tube length, and allowance is made for the refractive effects of the cover-slip. Modern objectives are usually corrected so that they work best with No. 2 cover-slips, which range in thickness from 0.17 mm. to 0.25 mm. Differences in cover-slip thickness may be compensated for by varying the tube length, increasing it for covers of less than standard thickness and decreasing it for those thicker than standard.

SUMMARY OF FOCUSING TECHNIC

Low Powers:

1. Adjust low-power objective (No. 3 or 16 mm.) 5 mm. above stage.
2. Arrange mirror so that clear white field is seen.
3. Place slide on stage (cover-slip upward).
4. Focus down with coarse adjustment until objective is within 3 mm. of the slide.
5. Focus upward with coarse adjustment until object is visible.
6. Regulate iris diaphragm.
7. Focus with fine adjustment to bring out details.

Changing from Low to High Powers:
1. Locate object with low power and place it in the center of the field.
2. Focus upward with coarse adjustment until objective is about 10 mm. above slide.
3. Turn high power objective into place.
4. Increase the diaphragm opening.
5. Focus downward with coarse adjustment until objective is about 1 mm. above the slide.
6. Focus upward with fine adjustment until specimen is apparent.
7. Regulate iris diaphragm.

Cautions:
1. Never focus downward with coarse adjustment while looking in the microscope.
2. Do not turn the fine adjustment more than five revolutions in either direction.
3. Do not change from low-power to higher-power objective without previously raising tube.
4. In case of accident do not attempt repairs or replacement.

Use of the Eyes.—Contrary to popular opinion, microscopical work is not injurious to the eyes. However, certain precautions must be observed to prevent discomfort and strain. Both eyes should be kept open, as the strain of squinting soon causes headache or tires the eye muscles. By keeping both eyes open, one eye is available for observation of the specimen and the other for transcribing the image to paper. For a few minutes, there will be difficulty in keeping both eyes open and in concentrating so that one obtains a clear picture with the eye used for observation, but this difficulty is very soon overcome. Still more difficult, but equally necessary, is the ability to use each eye for observation and to be able to change rapidly from one to the other. The proper distance of the eye from the ocular varies with oculars of different powers. The eye-point for low-power oculars is at a greater distance from the eye lens than is the

may be immediately turned into position and will be in approximate focus. Adjustments must be very accurate on par-focal objectives and the lenses can only be used on the instrument to which they have been fitted by the maker. As the lenses of high power objectives are smaller than those of low power, and admit less light, the diaphragm opening should be slightly increased. Lower the objective, using the coarse adjustment, until it almost touches the cover-slip, then slowly focus upward until the object is visible. A final regulation of the diaphragm is usually necessary; and the student should keep focusing with the fine adjustment so as to obtain good views of all parts of the specimen. As previously stated, the lens systems of microscopes are corrected for use with a certain tube length, and allowance is made for the refractive effects of the cover-slip. Modern objectives are usually corrected so that they work best with No. 2 cover-slips, which range in thickness from 0.17 mm. to 0.25 mm. Differences in cover-slip thickness may be compensated for by varying the tube length, increasing it for covers of less than standard thickness and decreasing it for those thicker than standard.

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5. Focus upward with coarse adjustment until object is visible.
6. Regulate iris diaphragm.
7. Focus with fine adjustment to bring out details.

Changing from Low to High Powers:
1. Locate object with low power and place it in the center of the field.
2. Focus upward with coarse adjustment until objective is about 10 mm. above slide.
3. Turn high power objective into place.
4. Increase the diaphragm opening.
5. Focus downward with coarse adjustment until objective is about 1 mm. above the slide.
6. Focus upward with fine adjustment until specimen is apparent.
7. Regulate iris diaphragm.

Cautions:
1. Never focus downward with coarse adjustment while looking in the microscope.
2. Do not turn the fine adjustment more than five revolutions in either direction.
3. Do not change from low-power to higher-power objective without previously raising tube.
4. In case of accident do not attempt repairs or replacement.

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case with those of high power. Cleanliness of lenses
and specimens, together with the proper regulation
of light, are important factors in saving the eyes from
undue strain.

Cleaning the Microscope.—Dust particles and
finger marks cause serious difficulty and annoyance
in working with a microscope. These foreign materi-
als are magnified just as much as the specimen and
thus result in indistinct images. Finger prints upon
optical parts are a sure sign of the careless worker,
and as they are easily avoided are all the more aggra-
vating. Slides and cover-slips should always be
handled by their edges, and the lens surfaces should
never be touched with the fingers. Finger prints
may be removed by breathing upon the surface show-
ing them, and quickly wiping it dry with cloth or
lens paper. Old smears not yielding to this treatment
may be removed with a cloth sparingly moistened with
alcohol or xylol; but the solvent must not be allowed
to penetrate the lens mounting. Dust particles on
lenses may be removed with a camel's hair brush or
dry soft cloth. By rotating the condenser, objective
and ocular, the position of dirt or other interfering
materials may be readily located. Cloudiness, especi-
ally upon the cover-slips of glycerin jelly mounts,
may be due to moisture, which may be removed with
a cloth moistened with alcohol. Dust on mechanical
parts is best removed with chamois or dry cloth.
Bearings and moving parts may be cleaned and lubri-
cated by wiping with a mixture of gasoline and light
machine oil. Fine adjustments are usually packed
with petrolatum. Solvents should not be allowed in
contact with lacquered parts. Discoloration of the

stage may be remedied by thorough washing and drying, followed by light application of paraffin oil all excess being removed by rubbing.

Interpretation of Images.—In viewing an object through the microscope one must remember that the image is inverted or reversed. Consequently, if one moves the slide toward the right, the object as viewed in the microscope will apparently move toward the left. If you desire to shift an object from the edge
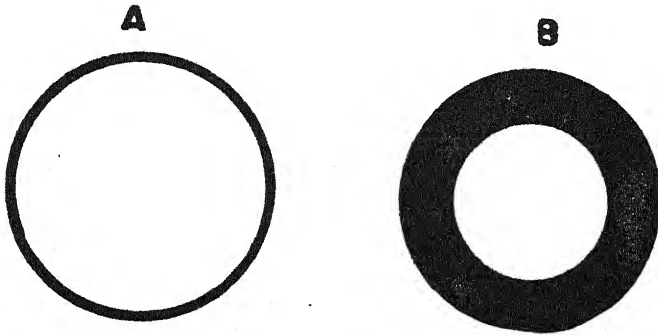
A. With diaphragm open.   B. With diaphragm partly closed

FIG. 25.—Air Bubbles.

to the center of the field, move the slide in the opposite direction. In working with temporary mounts the specimen should be allowed to stand for a few minutes before observation, as diffusion currents in the liquid cause rapid motion of the specimen. When viewing very small objects in temporary mounts, one will observe that they are in constant motion. This phenomenon is termed *Brownian movement*, and is exhibited by fine particles of solid materials in suspension or colloidal solution. In examining specimens having a curved or irregular surface, one must make

full use of the fine adjustment so as to gain clear views of the elevations and depressions in the specimen. The novice will often mistake air bubbles for cells or cellular material; but their behavior upon opening and closing the diaphragm serves to identify them. If, while looking at an air bubble, one closes the diaphragm opening, the wall of the bubble will increase in thickness, and if the diaphragm is opened, the wall will become thinner (Fig. 25). The walls of cellular elements are not affected in this way by the opening of the diaphragm.

Drawings.—The representation of microscopic images may be accomplished by photography, by the use of mechanical accessories and by free-hand drawing. The apparatus necessary for microphotography consists essentially of a microscope to which is attached the bellows and shutter of a camera. Separate lenses for the camera are not used, as the ocular serves to protect the image. While microphotographs give exact representations of the object, they are not suited for many purposes or for many types of specimens. The object must be specially prepared and one cannot, as is sometimes desirable, give prominence to one part of the specimen and repress others. It is difficult to combine in one photograph structures found in different fields. Mechanical accessories for delineation of microscopic images are far more useful than the microphotographic apparatus. The essential parts of the mechanical apparatus are a prism and a mirror so placed that the image produced by the ocular will be reflected upon a drawing surface. The reflecting device is so constructed that the worker may observe the specimen and at the same time

see the drawing surface. The apparatus is termed a *camera lucida* and is more carefully described in a subsequent chapter. With the camera lucida one can obtain drawings in which relative proportions are preserved, may combine in one drawing objects seen in different fields and may emphasize certain structures and ignore others.

Free-hand drawing of microscopic images requires patience and an aptitude which can only be acquired by practice. While natural talent for drawing is an advantage, it is not absolutely essential, and those not so talented will often produce more exact sketches. In sketching both eyes must be kept open and one eye should be at the microscope while the other is focused on the paper. Various parts of the specimen should be examined and only the clearest objects should be selected for drawing. One seldom finds satisfactory fragments of different tissues in a single field, and it is therefore necessary to explore a number of fields, selecting suitable materials from each and combining all in one drawing. Having decided upon the part of the specimen to be drawn, one should roughly plan it out on paper so that all parts of the object will be in proper relation both as regards size and position. The outlines should then be sketched in and finally light shading may be employed to indicate contrasts in depth of color. Particular attention is directed to the following points:

1. Each cell is entirely surrounded by a cell wall, and this is unbroken unless the specimen has been injured in preparation. In representing cell walls the lines should be continuous and should be brought back to the starting point.

2. A cell wall has a certain thickness and this must be repre-
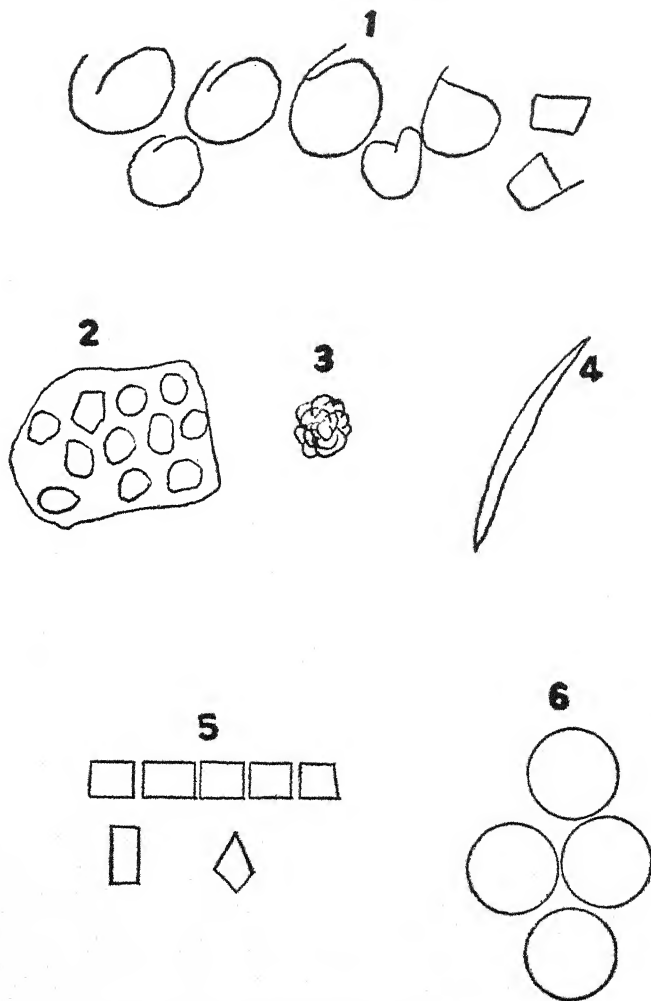
PLATE 26.—Common Errors in Sketching.

1. The cell walls of these parenchyma cells should be continuous and should be brought back to the starting point.   2. The cells in this fragment of tissue should be surrounded by a cell wall of definite thickness and the space between the cells is much exaggerated.   3. The parts of this aggregate crystal (rosette type) are sharp pointed and not rounded as illustrated.   4. The sides of this crystal should be straight lines as acicular crystals are never curved.   5. These cork cells are too symmetrical in form.   There are slight differences between individual cells and these are not represented.   6. Parenchyma cells never appear as exact geometrical forms.

sented by surrounding the cell with a double line or a
single line of substantial width.

3. Cells rarely appear as exact geometrical forms (circles, squares,
   oblongs, etc.) although they may closely approach these.
   There are slight but distinct differences between cells,
   even though they are adjacent. In a drawing, each cell
   should be fitted to the others in the tissue and the slight
   differences in shape and size should not be overlooked.

Broken or sketch lines should not be used, and
heavy shading should be avoided. Each drawing
should bear the name of the material from which
the slide has been prepared. The particular structure
which the drawing is intended to illustrate should
be noted in connection with the sketch.

CHAPTER IV

THE CHEMICAL REACTIONS OF PLANT TISSUES

WHILE great strides have been made in the realm of plant chemistry, much remains to be done. Although vegetable histology is more concerned with structure than with the chemical constitution of cell walls and cell contents, there are instances where specific reactions for the chemical substances present are of value in analytical work. Changes in the forms of cells are often accompanied by changes in their chemical nature. Thus, when the simple parenchymatic cell, the wall of which is composed of cellulose, is modified for purposes of support, a deposition of lignin in and about the cellulose wall takes place. But change in function is not always accompanied by change in chemical constitution, for the parenchymatic cells, if reinforced through the addition of extra layers of cellulose, will also perform the function of support. Whether or not the changes in structure of a given tissue are the result of chemical changes is problematical, but it appears more likely that change in function underlies both change in form and change in chemical constitution.

Many of the organic substances occurring in plants, either in the cell walls or in the protoplast, are so complex that exact chemical formulæ are not available. Despite all the study which has been given

to starch and the widespread occurrence and economic importance of this substance, a definite chemical formula has not been established for it. The same condition prevails with reference to many other plant substances. Therefore, in phytochemical work we are dealing with organic compounds which respond to definite chemical tests but to which we may not be able to assign exact formulæ. In dealing with the chemical constitution of plant tissues it is convenient to consider the reactions of the cell wall apart from those of the cell contents. This is all the more desirable because the compounds present in cell walls are relatively few as compared with those possibly present in the protoplasm and those which result from the physiological activity of the latter.

GENERAL METHODS

The application of microchemical reagents to sections of plant parts and to powdered material is generally a very simple procedure, and perhaps the most important factor in securing good results is that of reaction time. The length of time for which a given specimen should be exposed to the reagent is likewise the most variable factor and depends upon concentration of the reagent, thickness of the specimen, amount of the specific constituent present, and the nature of the reagent. In general, sectioned material is used in demonstrating chemical constitution of cell walls and protoplasmic contents. Powdered materials, owing to the disintegration of the cells and consequent liberation of the non-protoplasmic contents, are more likely to be used in demonstrating starch and the other non-protoplasmic cell

contents.   It must be borne in mind that the following notes are of general application only and that each reagent shows individual peculiarities which often can only be determined by experiment.

In dealing with sectioned materials the reagent may be applied directly to the sections as they come from the microtome, either with or without removal of paraffin, as the latter substance may be considered inert.   If it is desirable to remove the paraffin, this may be done by washing the sections in xylol or chloroform, followed by thorough removal of the solvent by spontaneous volatilization or by gentle heat.   Removal of the paraffin by heating with water is not recommended as, aside from the possibility of changes in chemical composition through boiling, the sections will hold a certain amount of water, thereby diminishing the speed of the reaction through dilution of the reagent, and in some instances the water acts as an interfering substance.   Small porcelain evaporating dishes or Petri dishes may be used for holding the sections and, where one is dealing with a single section, the hollow slides used in bacteriological work may be convenient.   In some instances the sections may be attached to an albumin-coated slide, the paraffin removed by xylol, and the reagent applied after evaporation of the latter.   While this procedure is more likely to preserve the sections intact, it can only be used where there are no possibilities of interaction between the reagent and the albumin-glycerin coating.

In applying microchemical tests to powdered materials it is often advantageous to note the effects of the reagent as it comes in contact with the par-

ticles of the substance under examination. This is best accomplished by mixing the powder with water, placing a cover-slip on the preparation, and removing excess water by touching a piece of filter paper to the edge of the cover-slip. A few drops of the reagent are now placed in contact with the edge of the cover-slip, and if a piece of filter paper is placed at the opposite side the reagent will be drawn under the cover-slip to replace the water removed by the filter paper. This procedure can only be used with reagents miscible with water and not losing their activity through dilution. If the reagent is miscible with water and its activity is not too greatly impaired by dilution, it is always better to mix the powder with water so as to secure even distribution and consequently more uniform action of the reagent upon the particles. If, however, the reagent is immiscible with or inactive in the presence of water, it is applied directly to the dry substance and thoroughly mixed. Excess of reagent can be absorbed by strips of filter paper if a cover-slip be placed upon the specimen and the paper touched to the edge of the cover. In dealing with large quantities the reagent may be added directly to the powder, and the mixture well shaken and set aside to settle, or for more rapid separation, centrifuging may be employed.

CHEMICAL PROPERTIES OF CELL WALLS

The walls of primitive cells are usually composed of cellulose; but in the building up of more complex structures the cell walls undergo more or less change in chemical composition as well as in appearance.

The substances most frequently occurring in the cell walls are cellulose, lignin, cutin, suberin and gums, including pectinous substances. Many of the aniline stains give characteristic colorations with these substances, and these reactions will be considered in the section on Staining.

Cellulose.—This is a carbohydrate to which the empirical formula $(C_6H_{10}O_5)_x$ has been assigned. The following reactions may be used in the identification of cellulose:

1. A blue coloration with iodine and sulphuric acid. Treat the material with iodine-potassium iodide solution, then add a few drops of sulphuric acid (70 per cent).
2. A violet coloration with zinc chloriodide solution. Apply the reagent directly to the material.
3. Solubility in cuprammonia solution. Apply the reagent directly to the material.

Lignified Walls.—It is possible that lignification of cell walls is a deposition of woody materials upon the cellulose membrane. This view is borne out by the fact that lignified cell walls that have been treated to remove the lignin respond to tests for cellulose. The existence of a definite substance termed *lignin* is extremely doubtful and it is probable that the reactions given by lignified walls are due to several compounds occurring in woody materials. Among these compounds are lignic acids, wood gum, coniferin, vanillin, and other aromatic aldehydes. Lignified membranes or cell walls are found in those tissues of the plant which act as supporting elements or which require support. The following reactions may be used in the microchemical identification of lignified cell walls:

1. A yellow or brown coloration with iodine and sulphuric acid. Treat the material with iodine-potassium iodide solution, then add a few drops of sulphuric acid (70 per cent).
2. A yellow or brown coloration with zinc chloriodide solution. Apply the reagent directly to the material.
3. A red or violet coloration with phloroglucin solution. Apply the reagent simultaneously with concentrated hydrochloric acid.

Cutinized and Suberized Walls.—The substance *cutin* occurs in the epidermal tissues, especially, in those of the green parts of the plant. These cutinized walls form a thin, transparent, waterproof membrane or covering tissue, which protects delicate structures from injury and excessive evaporation of water. As the plant matures the epidermal tissues of the root and stem are gradually replaced by cork, which contains the substance *suberin* in the walls of its cells. Many of the microchemical reactions of cutinized and suberized walls indicate that fatty substances may be present; but that these are not comparable to our common fats is proven by the fact that the usual solvents for fats have no effect on these tissues. Corky or suberized cells protect the mature plant against temperature changes and mechanical injury. Cutinized and suberized cell walls give similar reactions with most reagents, and for differentiation we must depend upon structure and location rather than upon chemical properties. The following reactions are chiefly of use in distinguishing between cellulose or lignin and cutin or suberin:

1. Concentrated potassium hydroxide solution (30 per cent) produces a yellow to brown coloration in suberized walls. Apply the reagent directly to the material and heat gently.
2. Concentrated alcoholic chlorophyll solution, acting in the dark, stains cutinized and suberized walls green, but does

not affect cellulose or lignified walls. The reagent must be freshly prepared and should remain in contact with the material at least thirty minutes.

3. Alkannin solution colors cutin and suberin red. This reaction requires several hours and the red color is not as deep as with fats.

Gums and Gelatinized Walls.—The cell wall in certain plants is so modified in its chemical nature that the original cellulose has been more or less replaced by substances which, in contact with water, behave like gums and form mucilages. Gums may also occur in the coats of certain seeds, their function being to attract water from the soil, thus promoting germination. The majority of gums are of carbohydrate nature with a percentage composition similar to that of cellulose $(C_6H_{10}O_5)_x$ but differing from this substance in chemical properties. Because of their varied chemical characters it is impossible to state general tests for gelatinized cell walls and the gums. The following tests, excluding the first, are only applicable to certain tissues and products of this class:

1. The fact that gums swell when brought into contact with water is of value in demonstrating the presence of these substances in the cell wall. The material should be mounted in absolute alcohol and the water gradually drawn under the cover-slip.
2. Blue coloration, similar to that of cellulose with iodine or with iodine and sulphuric acid.
3. Violet coloration with zinc chloriodide.
4. Partial or complete solubility in cuprammonia solution.
5. Corallin dissolved in 30 per cent sodium hydroxide colors certain gums red.

The secretion of gum (gummosis), occurring around wounds in certain plants, is probably an effort to seal the abrasion and thus prevent injurious fungi and

bacteria from reaching vital parts. It is noteworthy that this wound gum does not swell upon the addition of water and that it responds to many of the tests for lignin.

Fungus Cellulose (Chitin).—The cell walls of fungi, while extremely thin and similar to cellulose walls in appearance, are of different chemical composition and do not respond to microchemical tests for cellulose. Chitin (Iwanoff), a substance occurring in certain animal tissues, is perhaps the chief constituent of the cell walls of fungi. The following microchemical test (Viehoever) is based upon the conversion of chitin to chitosan and has given excellent results in the detection of mold in foodstuffs:

> Heat the material to about 90°C. with 50 per cent sodium hydroxide for one hour; decant or centrifuge to remove the alkali liquid; wash with alcohol to remove all traces of alkali; treat with iodine-potassium iodide (2 iodine, 1 potassium iodide in 200 cc. water) until walls are stained; pour off excess iodine solution and apply sulphuric acid 1 per cent. The fungus cellulose assumes a red to violet color.

Pectinous Substances.—Pectins, or mixtures of pectic substances, include pectin, pectose, and pectic acids and occur in the walls and in the cellular spaces of many plant tissues, especially where lignin and cutin are absent. These substances are responsible for the hardening or "jelling of jellies." The following reactions for this class of materials are possibly more in the nature of staining properties than of chemical tests.

> 1. Pectic substances are best identified by their reactions with various aniline dyes. Fuchsin, methylene blue, and Bis-

marck brown stain pectic materials as well as lignin and suberin; but the latter substances retain the color after treatment with alcohol or acids, whereas the pectic substances are decolorized by this treatment.

Inorganic Wall Substances.—Many cell walls contain more or less inorganic material. This can best be demonstrated by carefully ashing the section in a furnace free from air currents. The heating must be done upon a silica or quartz plate. Great care is necessary in removing the plate after ashing and the ash must be examined dry. In sections of Equisetum stem, the amount of inorganic material in the cell walls is so great that a perfect counterpart of the specimen will remain after this treatment. .

CHEMICAL PROPERTIES OF CELL CONTENTS

The protoplasm, or material within the wall of a vegetable cell, contains structures concerned in the physiological activity of the cell together with *inclusions*, or substances produced by this protoplasm. The term *cell contents* might be used for all materials within the cell wall, thus including such physiological parts as the nucleus and vacuoles, together with starch, inulin and other inclusions. However, in vegetable histology the term *cell contents* is restricted to materials resulting from the activity of the protoplasm and would therefore include starch grains, calcium oxalate crystals and other substances. These inclusions, or nonprotoplasmic cell contents, may be inorganic or organic substances. They are most conveniently subdivided into substances of definite form and those of indefinite form. The histology of the various cell contents will

be considered in a subsequent section but their chemical properties are briefly noted under the following headings.

Starch.—The empirical formula assigned to starch is $(C_6H_{10}O_5)_x$; but there are differences of opinion as to whether the starch grain is homogenous in structure and whether it is a simple substance or a mixture. Starch is the most widely distributed of all the materials classed as cell contents, and in amount exceeds all others. Starch grains swell, upon the addition of boiling water, and form a pasty mass. Complete solution of the grains is not affected unless they are treated with superheated steam. Solutions of alkali hydroxides even at moderate temperatures cause formation of a paste with swelling of the grains. Roasting transforms starch to dextrins which are more or less soluble in water. The starch granules of mace and certain other plants will be colored red instead of blue when treated according to the test given below. Such grains have probably been partially dextrinized. The following reaction is used in the identification of starch:

1. Addition of dilute aqueous solution iodine-potassium iodide colors starch grains blue. This reagent is best added at the edge of the cover-slip and drawn under by touching filter paper to the opposite edge, as in this manner one can observe the gradual coloration of the starch grains as the iodine comes in contact with them. Heating causes a disappearance of the color, but it reappears upon cooling. Very characteristic results may be obtained by the addition of chloral hydrate solution to specimens previously treated with the iodine solution. The chloral acts as a clearing agent, destroys many of the cell contents and finally causes swelling and disintegration of the starch grains. Demonstration of starch in large pieces of tissue may be accom-

plished by placing the plant part in a concentrated chloral hydrate solution containing iodine, and heating the mixture to near boiling.

Inulin.—Inulin has the same empirical formula as starch and, as it is readily soluble in water, occurs in solution form in plant tissues. It can be precipitated in plant cells by the addition of alcohol, but dissolves readily upon the subsequent addition of water. The following reactions may be used in the identification of inulin:

1. Addition of aqueous iodine-potassium iodide solution colors inulin deposits brown.
2. Pyrogallol solution colors inulin violet red.
3. Acetic acid dissolves inulin, producing a greenish solution.

Sugars.—The chief sugars found in plants are glucose, levulose and sucrose (cane sugar). The empirical formula for glucose and levulose is $C_6H_{12}O_6$, while that of sucrose is $C_{12}H_{22}O_{11}$. The sugars occur in solution in the living plant tissues and, like inulin, they may be precipitated by the addition of alcohol. The general reactions for the identification of sugars in plant cells are as follows:

1. Addition of alpha-naphthol solution to the sections, followed by a few drops of concentrated sulphuric acid, yields a violet coloration within a few minutes. Inulin also responds to this test.
2. Treat the section with copper sulphate solution (20 per cent), wash, add alkaline Rochelle salt solution (potassium hydroxide 10 gms., Rochelle salt 10 gms., in 100 mils of water) and boil. Cuprous oxide will be precipitated in cells containing sugars and will appear black by transmitted light and red by dark field illumination.
3. Many sugars form characteristic crystalline osazones upon treatment with phenylhydrazine in the presence of acetic acid. The sections should be boiled for at least one hour with the phenylhydrazine solution and then rapidly cooled.

Unless thick sections are employed the sugars will be dissolved in the reagent and the precipitate will form at the bottom of the liquid rather than in the individual cells. The phenylhydrazine solution is prepared according to the formula noted in the Appendix.

Alkaloids.—Alkaloids are basic compounds containing carbon, hydrogen, nitrogen and oxygen, although the latter element is wanting in the liquid alkaloids. The toxic effects upon animals and plants are of great interest, and the salts of these alkaloids are among the most important items in our materia medica. The alkaloids, being basic in reaction, readily form salts with the acids present in the plant cells, and these alkaloidal salts present in the plant are usually in solution, although amorphous deposits of alkaloidal tannate are present in some drugs. The microchemical reactions of alkaloids are often complicated by interfering substances which seriously impair the value of tests. The general tests for alkaloids in plant tissues are as follows:

1. Addition of a few drops of mercuric-potassium iodide solution to the section causes the formation of a yellowish flocculent precipitate within the cells containing alkaloids.
2. Iodine potassium iodide solution similiarly applied yields reddish or brownish precipitates with nearly all alkaloids.
3. The alkaloids of Hydrastis may be precipitated *in situ* by mounting freshly cut sections in sulphuric acid.
4. Extract the material with ammoniated ether (ether saturated with ammonia by shaking 100 cc. ether with 5 cc. concentrated ammonia water and separating the ether from the excess ammonia water), neutralize with dilute hydrochloric acid, carefully evaporate the separated acid solution to dryness, dissolve in a few drops of water. Upon the addition of gold chloride or platinic chloride (1 per cent solutions) many alkaloids yield characteristic crystalline precipitates, and practically all alkaloids respond to the reagents used in Tests 1 and 2,

Glucosides.—The glucosides are plant principles which, when decomposed by dilute acids or enzymes, yield sugar (chiefly glucose) as one of the products of the reaction. Aside from this property of yielding glucose upon decomposition there are no group reactions which may be used in their identification. A few glucosides have the property of reducing copper sulphate directly, or without apparent decomposition and subsequent production of glucose. Many of the glucosides, upon hydrolysis or decomposition, yield sugars which give characteristic crystalline precipitates with phenylhydrazine.

Calcium Oxalate.—The great majority of crystals occurring naturally in plants are composed of calcium oxalate. This substance crystallizes either in the monoclinic or in the tetragonal system, but the crystals naturally occurring in plants are rarely symmetrical. In many plants the calcium oxalate occurs in small broken crystals termed *crystal sand*. The tests for calcium oxalate are as follows:

1. Nitric and hydrochloric acids dissolve the oxalate crystals.
2. Concentrated sulphuric acid causes destruction of the oxalate, resulting in the formation of insoluble needle crystals of calcium sulphate.

These tests are best performed by adding a few drops of the acid to the edge of the cover-slip and drawing under by application of a filter paper at the opposite edge of the cover-slip.

Aleurone.—Aleurone is a protein or nitrogenized principle found in many seeds, especially those rich in fixed oil. Except in the cereals, it seldom occurs associated with starch. Aleurone is present in the form of granules surrounded by a membrane enclosing

a protein substance in which may be embedded globular particles (globulins), protein crystals (crystalloids) and calcium oxalate crystals. Very little is known regarding the exact composition of these proteins and their formation in the plant. The general tests for aleurone and other plant proteins are as follows:

1. Iodine-potassium iodide solution colors proteins yellow to brown. The iodine solution should be more concentrated than that used in the test for starch and is best added at the edge of the cover-slip and drawn under by applying filter paper to the opposite edge of the preparation.
2. Concentrated nitric acid, applied as in Test 1, colors proteins bright yellow (xanthoproteic reaction).
3. Millon's reagent gives a bright red color upon warming the material to which the reagent has been applied.

Tannins.—The tannins are a group of chemically allied substances chief among which are tannic and gallic acids. In the living plant they occur in solution or as amorphous deposits. Tests for tannins in vegetable materials are as follows:

1. Ferric chloride solution gives a bluish or greenish black coloration, depending upon the particular tannin compound present.
2. Cupric acetate solution produces a reddish brown precipitate with tannins.

These tests may be performed by adding the reagent at the edge of the cover-slip and securing gradual diffusion by applying filter paper to the opposite edge of the cover.

Resins.—The resins include a complex group of substances which are found in many plants. In a number of instances volatile oils or gums are associated with these resins, thus forming *oleoresins* and

gum resins. Owing to the great variation in chemical composition and the scarcity of definite information upon the resins, there are but few general micro-chemical tests for this group.

1. The resins present in sections of plant organs will generally dissolve upon treatment with concentrated alcohol.
2. After acting for several hours, alkannin stains resin deposits red.
3. Cupric acetate solution (35 per cent) stains resin deposits an emerald green. This reaction only occurs upon exposure of the sections for not less than six days to the action of the copper solution.

Silica.—Silica, silicic acid, or silicon dioxide, may occur as deposits upon the walls of cells or in the form of rounded masses. This substance is only soluble in hydrofluoric acid and is not destroyed by heating at high temperatures. The following tests may be employed:

1. Retention of form after heat treatment on a quartz slide, as directed in a previous section dealing with inorganic wall substances (page 68).
2. Insolubility in concentrated mineral acids with the exception of hydrofluoric acid. As the latter acid attacks glass the test is best performed upon transparent pieces of celluloid, and the microscope objective must be protected by attaching a cover-slip to the end lens by glycerin or immersion oil.

Calcium Carbonate.—Calcium carbonate occurs free in the plant cells, as deposits upon the cell walls, or in the form of irregular masses attached to the cell wall by a slender filament of cellulose.

1. Addition of concentrated hydrochloric acid dissolves deposits of calcium carbonate with the evolution of gas bubbles (carbon dioxide). The acid may be placed at the edge of the cover-slip and drawn under, as in previous tests.

Fats.—The fats and fixed oils are organic salts of the so-called fatty acids, chiefly palmitic, stearic, and oleic acids. They usually occur in the form of globules within the living cells of seeds and fruits. They are rarely associated with starch but often occur in seeds containing aleurone. The microchemical tests for oils and fats are as follows:

1. Addition of alkannin solution colors fat and oil globules bright red. Suberized walls are also colored red by this reagent. The reagent should remain in contact with the specimen for at least thirty minutes.
2. Boiling with concentrated potassium hydroxide solution or strong ammonium hydroxide causes saponification, which is evidenced by a loss of refractive power in the globules. Upon cooling, the globules are found to be covered with small needle crystals of soap. Water must be added during the heating, and the reagent is added directly to the specimen.

CHAPTER V

STAINING

THIN sections of light-colored specimens, especially those prepared by the infiltration method, are apt to be so transparent that the details of structure are almost invisible. The difficulty is overcome by coloring or staining the sections with various dye-stuffs. The staining process is either a saturation of the cellular material with the color, or a chemical reaction between the stain and the compounds contained in the cell. In the latter instance the stain plays the part of a reagent. As a rule the nuclear material stains more readily than the other parts of the cell; therefore upon the application of a *saturation* or *general stain*, the nucleus will always appear more deeply colored than the surrounding parts.

With comparatively few exceptions the stains in use are aniline colors and are kept in the form of saturated alcoholic solutions. These solutions should be kept in glass-stoppered bottles in a dark cool place. Unless otherwise directed staining solutions are prepared by diluting 5 mils of the alcoholic solution with distilled water, to make 100 mils. If sections are in aqueous media and not attached to slides, they may be stained by placing them in a shallow dish filled with the stain. If the sections are dehydrated, whether attached to slides or free, they must be

rehydrated as directed in a previous chapter. The time required for staining varies with the material and the stain employed, and proper conditions can only be determined by experiment. The general statement may be made that most vegetable sections will be sufficiently stained in ten minutes. For ready manipulation of specimens attached to slides there are several forms of special staining jars available. These jars have a decided advantage in that the slide is placed on edge during the staining process, thus avoiding deposition of foreign materials. They are also economical in that less staining fluid is required. These jars are usually provided with covers and may be used as laboratory containers for stains in frequent use. Where large numbers of specimens are to be stained, staining racks are more desirable than jars. These racks accommodate as many as twenty-five slides and are accompanied by a tray in which the stain may be placed.

In many instances better results and more exact control may be had by overstaining and subsequently washing out the excess stain with dilute alcohol.

Combinations of two or more stains may be used for the purpose of obtaining differences in color in different parts of the cell. This process is termed *double staining*. Double staining may be effected by using combinations of different dyes in one solution or by using a separate solution of each stain. Certain cell substances, notably those of many micro-organisms possess the power of retaining the stain after immersion in dilute hydrochloric or sulphuric acids. This firm combination of stain and cell substance is

termed *acid-fast*. Although this character is of minor importance in vegetable histology it is of great value in differential work on organisms. Specimens should be well rinsed in water after all staining procedures.

Staining Technic.—Stains are more advantageously applied to sectioned specimens than to powdered materials; and, in dealing with sectioned materials, the following three procedures are at our disposal:

1. *Mass Staining.*—This method is only applicable to very small portions of material, and the latter must be fairly homogeneous and readily permeable to the staining fluid. Unless these conditions be fulfilled it is difficult to secure even staining throughout the fragment. Another disadvantage is the likelihood of the stain being removed in subsequent operations, especially in dehydration. In fact, this procedure only gives satisfaction where comparatively few sections are to be prepared by the non-infiltration method. The material is placed in the staining fluid for times varying from one to twenty-four hours, depending upon its permeability.

2. *Staining of Attached Sections.*—This is the best method for staining very thin specimens, as handling is reduced to a minimum. After removal of the paraffin and evaporation of the xylol the slides are placed in 95 per cent alcohol for ten minutes, followed by 80 per cent alcohol for five minutes, and finally brought into 70 per cent alcohol for five minutes. They are now sufficiently rehydrated for staining purposes and, after the excess alcohol has been drained off, may be placed in the staining fluid. It is well to examine a test slide every five minutes so as to secure best results. After staining is complete the specimens are again dehydrated and mounted in Canada balsam as directed in Chapter I.

3. *Staining of Loose Sections.*—This method is convenient for staining sections prepared by the non-infiltration method if the material is not too delicate to stand handling. It may also be applied to infiltrated specimens after rehydration. In either case the specimens are drained free of water by being placed in a piece of fine wire mesh; this mesh is then lowered into the staining fluid for action of

the latter. Test specimens should be examined at intervals, and when staining is complete the mesh is lifted from the staining vessel and excess stain removed by placing the mesh in a vessel with water. The washing should be continued until the water is but slightly colored by the stain.

Stains for Cell Walls.—Differences in chemical composition of cell walls and contents are rendered more apparent by the application of reagents and stains. The color changes incident to the action of reagents are due to chemical interaction, whereas, with stains the coloration is usually due to a saturation of the cellular material by the dye. It is noted that certain tissues color easily with a green stain, while others are indifferent to its action. Certain stains may thus be used to differentiate cellular elements from others closely resembling them. Stains for this purpose are termed *differential stains* in contradistinction to *general stains*, which color most, if not all, tissues. The effects of differential stains are somewhat analogous to the actions of reagents. While the various substances present in the walls of cells respond to both reagents and stains, the cell contents for the most part are identified by their reactions rather than by their staining properties. Certain cell contents may color with a given stain, but the process is more of a general absorption than a specific character. It is for this reason that only the staining properties of cell walls have been included in this work. Formulæ for the staining fluids recommended in the following procedures will be found in the Appendix.

Cellulose:

Delafield's hematoxylin:

Cellulose walls are colored violet. The time required is five to fifteen minutes.

Aniline blue and methyl blue:

Cellulose walls are stained an intense blue in about one hour. The color is not removed by alcohol or the usual clearing agents.

Congo red:

Saturated aqueous solutions stain cellulose bright red. Time required is twenty-four hours. Sections should be washed in alcohol after staining and are best mounted in Canada balsam.

Lignified Tissues:

Delafield's hematoxylin:

Lignified walls are colored yellow to brown. The time required is at least thirty minutes and usually longer, depending upon the texture of the material.

Fuchsin:

Lignified walls are stained deep red. Aqueous solutions are used and the time required is fifteen to thirty minutes. Best results are obtained by washing the stained material for five to ten minutes in Altman's picric acid solution.

Double staining may be had by first using fuchsin, washing in alcohol for one hour, and then staining with hematoxylin, aniline blue or methyl blue. By this procedure cellulose walls are stained violet with hematoxylin or blue with the blue dyes, while the lignified tissues stain red with the fuchsin.

Cutinized and Suberized Tissues:

Cutinized cell walls stain slowly and with difficulty. Cyanin, saturated solution in 50 per cent alcohol, stains cutinized membranes deep blue. Sections are partially decolorized in Javelle water and an equal volume of glycerin is mixed with the cyanin. The time required is at least twenty-four hours.

Aniline water safranin stains suberized walls yellowish and lignified walls blue. Allow the stain to act for at least thirty minutes, wash in acid alcohol, then in alcohol, until washings are colorless.

Alkannin stains cutinized and suberized walls red.

Double staining may be accomplished by washing sections stained in aniline safranin with alcohol and then staining

with concentrated aqueous methyl blue for at least fifteen minutes.   The blue stains cellulose walls.

Gums:

While mucilaginous cell walls do not stain satisfactorily, a mixture of equal parts alcoholic fuchsin and methyl violet, according to Hanstein, often gives good results.

Pectins:

Fuchsin stains pectic substances, lignified tissues, and suberin, deep red.   When the stained specimens are washed with acid alcohol, the pectic substances are decolorized, while lignin and suberin retain the color.   The time required is between fifteen and thirty minutes.

CHAPTER VI

PLANT CELLS AND TISSUES

General Considerations.—The fact that a plant is an aggregation of small bodies or cells was first brought to general attention in 1667 by Robert Hooke, an English lens manufacturer. This discovery was merely incidental to the attempts then being made to improve the crude lenses of the time; and Robert Hooke used plant sections merely as demonstration objects. Grew and Malpighi, working independently, confirmed Hook's observations, and their published researches are the earliest records of work upon the cellular structure of plants and animals. All living bodies are composed of one or more small units or cells. The simplest organisms consist of but one cell (*unicellular*), all functions necessary to the life and continuance of the species being performed by this individual unit. The more complex organisms begin life as a single cell, but this unit rapidly undergoes division and forms a *multicellular* individual. In the unicellular organism all life processes are performed by a single cell; but, fairly early in the development of multicellular plants and animals, there occurs a differentiation of cell structures resulting in the formation of tissues. A *tissue* is a group of cells of similar structure and is designed to perform a certain work for the organism. The work that a tissue performs

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is termed its *function*, and the existence of a multicellular organism is dependent upon the proper functioning of its component tissues.

Cytology, or the study of cells, reveals many structures within each cell, and it is customary to consider a cell as consisting of a nucleus, a cell wall and the
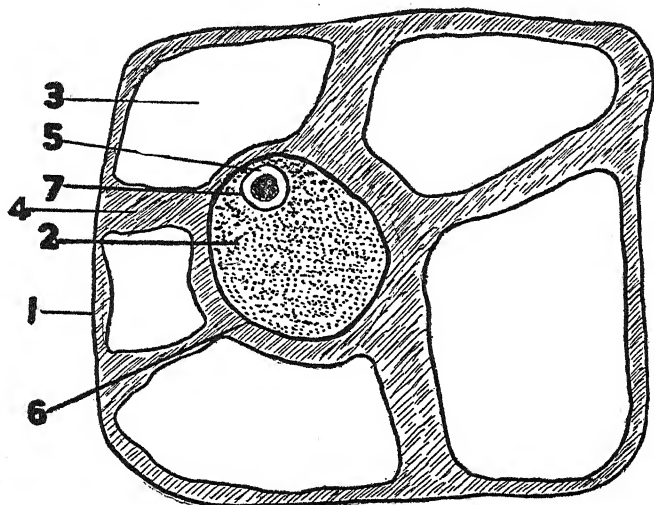
PLATE 27.—Plant Cell from Tradescantia Stem.

1. Cell wall.   2. Nucleus.   3. Vacuoles.   4. Cytoplasm.   5. Nucleolus.
6. Nuclear membrane.   7. Nucleolar membrane.

cytoplasm, or material within the wall. The study of cell walls and their modifications is a subject of great importance in that part of vegetable histology dealing with the microanalysis of foods and drugs. The *cytoplasm* is a semi-solid substance in which the presence of several structures, or cell contents, can be demonstrated by proper treatment (Plate 27). These structures may be classified as *protoplasmic con-*

tents, or those responsible for the life of the cell, and the *non-protoplasmic contents* or *inclusions,* substances resulting from the activity of the protoplasmic contents. The protoplasmic contents include the *nucleus, chromatophores* or *plastids,* and *chondriosomes.* The nucleus is separated from the body of the cytoplasm by a thin membrane and contains a granular substance termed *chromatin,* together with delicate thread-like structures termed *linin threads,* and one or more spherical bodies termed *nucleoli.* The chromatophores are small bodies embedded in the protoplasm and are of importance in the manufacture of the non-protoplasmic contents. According to location and specific function, the chromatophores are colored (*chromoplastids*) or colorless (*leucoplastids*). The green color of leaves is due to the presence of green *chromatophores* or *chloroplastids* and, as will be noted in a subsequent section, these are directly concerned in the production of starch by the plant. The chondriosomes are exceedingly small granules or rods occurring in the cytoplasm, and their function is not definitely known. The position occupied by the nucleus in plant cells, and the appearance of the protoplasm depend upon the age of the cell. In newly formed cells, or those in parts of the plant where growth is active, the nucleus is in the center of the cell and the latter is entirely filled with protoplasm. In mature plant cells, or those located in parts of the plant not active in growth, the protoplasm undergoes shrinkage and cavities or *vacuoles* are formed. As the cell grows older and less active, the vacuoles increase in size until the remaining protoplasm is merely a narrow strip in contact with the cell wall. The vacuoles are rapidly filled

with a liquid termed *cell sap*, which is of importance in the distribution of nutrients to the growing portions of the plant. The nucleus always remains embedded in the protoplasm, and when the latter has been reduced to a layer in contact with the cell wall the nucleus will be moved toward this wall. In very old cells, such as the wood in the center of a tree, all traces of protoplasmic contents are lost and the cells are in reality dead. The walls and cavities of these dead cells perform important functions as supporting and transporting elements.

The non-protoplasmic contents or inclusions comprise a large number of substances and these show great variation both as regards chemical composition and structural character. A majority of plants contain starch, a typical inclusion; but the granules of starch in different plants show material differences in structure. The non-protoplasmic contents include alkaloids, enzymes, starch, glucosides, sugars, fats and many other substances. In dealing with foods and drugs one seldom encounters the nuclei or other protoplasmic contents; therefore, in microanalysis the term cell contents is generally applied to the non-protoplasmic cell contents.

Structure of the Cell Wall.—The walls of growing cells are mere membranes composed of the substance *cellulose* with a thin layer of pectinous substance, the *middle lamella* (Plate 48), intervening between adjacent cells. Upon attaining full growth, many cells require a stronger covering, and this is obtained by a thickening of the original membrane. Thickening of a cell wall may be accomplished by the deposition of successive layers of cellulose or other materials

outside the original membrane, or by the introduction
of substances between layers of cell wall already
formed.   Thickening by deposition is the usual method
and is termed growth by *apposition*.   Introduction
of substances between layers of wall is termed growth
by *intussusception*.   In growth by apposition the
different layers may be clearly marked or may be
so consolidated that but a single homogeneous wall
is apparent.   Growth by intussusception is often
apparent in walls that show thickenings at certain
points and not at others.   Although the primitive
cell membrane is composed of cellulose, this substance
is usually replaced by other materials during the
process of growth in the cell.   Lignified cell walls
are those in which a woody substance has been de-
posited upon the cellulose membrane.   Suberized cell
walls are found in cork cells, and result from the depo-
sition of suberin upon the cellulose.   Cutinized walls
occur in the covering membranes of leaves where
the original cellulose has been replaced by cutin.

ORIGIN OF CELLS AND TISSUES

Every plant, unicellular or multicellular, is the
direct descendent of a preceding generation.   In the
lower forms of plant life the production of new cells
is often a comparatively simple process and consists
in a direct division of the nucleus followed by the
formation of a wall between the divided nuclei.   The
wall between the cells is formed by a constriction of
the original cell wall and an inward projection of the
latter, until the newly formed cell is separated from
the parent cell.   In certain lower plant forms, the
formation of a cell wall after the nuclear division
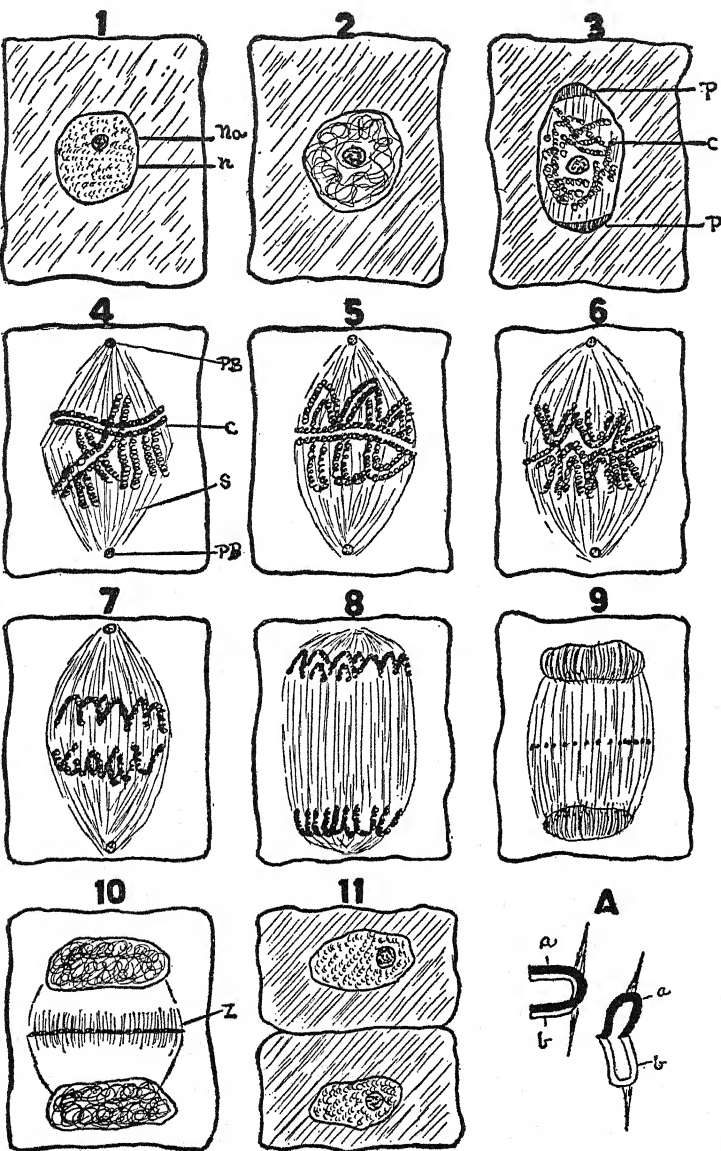
PLATE 28.—Changes in a Cell during Mitosis. (Modified from Strasburger, Noll, Schenck, Karsten, "Text Book of Botany.")

No, Nucleolus. *N*, Nucleus. *P*, Polar caps. *C*, Chromosomes. *PB*, Polar centers. *S*, Spindle fibers. *Z*, Cell plate. *A*, Separation of chromosome loops by fibers of the spindle. *a* and *b* are halves of a chromosome loop.

is not accompanied by separation of the cells from each other. In the higher forms of plant life the process of cell division is extremely complex and is termed *mitosis* or *indirect nuclear* division. Mitosis, like the direct method of cell reproduction, consists essentially in a division of the nucleus followed by the formation of a cell wall between the separated nuclei, but differs in the manner in which the nuclear division is accomplished. The details of mitosis or indirect nuclear division are but briefly described in the following statements. The network of linin threads is unraveled into a definite number of semicircular filaments or loops, each of which is covered with chromatin bodies. These filaments are now termed *chromosomes* and are so arranged that the curved portion of each is toward the center of the nucleus. Simultaneous with this arrangement of the chromosomes is the development of two series of fibrils (spindle fibers) extending from opposite sides of the nucleus and having their origin in a slightly differentiated nuclear material, the *polar caps*. One set of these fibrils extends completely across the nucleus, while the fibrils of the second set extend between the polar caps and the chromosomes. Contraction of the fibers of this latter set effects a longitudinal separation of each chromosome into two segments (Plate 28, A) and the respective chromosome segments are drawn toward opposite poles of the nucleus. The group of chromosomes at each polar cap becomes invested with a membrane, and each group forms the nucleus of a cell. Formation of walls to surround the cells, of which each of these nuclei forms the center, follows soon after separation of the chromosomes. The

forerunner of the new cell wall is formed at certain points by a thickening of the fibrils extending between the polar caps. These thickened portions form a structure which extends across the cell and which is ultimately covered by layers of cellulose produced by the cells that have just undergone division. The new cells are furnished with chromoplastids by direct division of these present in the parent cell.

PLANT TISSUES

Immediately upon fertilization of the egg-cell contained in the ovule, multiplication of cells by the process of mitosis occurs, and an embryo is formed. In the earliest stages of embryo formation the cells produced by indirect division are alike in structure, but a differentiation of cells soon occurs in the development of the embryo. This differentiation ultimately results in the formation of tissues, or groups of cells having specific characters. As previously noted, each tissue has a certain function and is particularly fitted to perform this function by certain peculiarities in the structure of its cells. In the young embryo practically all the cells are capable of division (Plates 34, 35); but in the mature plant this property is present in comparatively few cells and these form the *meristematic tissues* located at the growing points of stems and between the bark and wood of stems and roots. The young embryo may be divided into three zones of more or less differentiated cells, an outer or *dermatogen region* from which the first covering tissues of the plant are derived; a middle or *periblem region* from which the covering tissues of the mature plant are derived; and a central or *plerom*
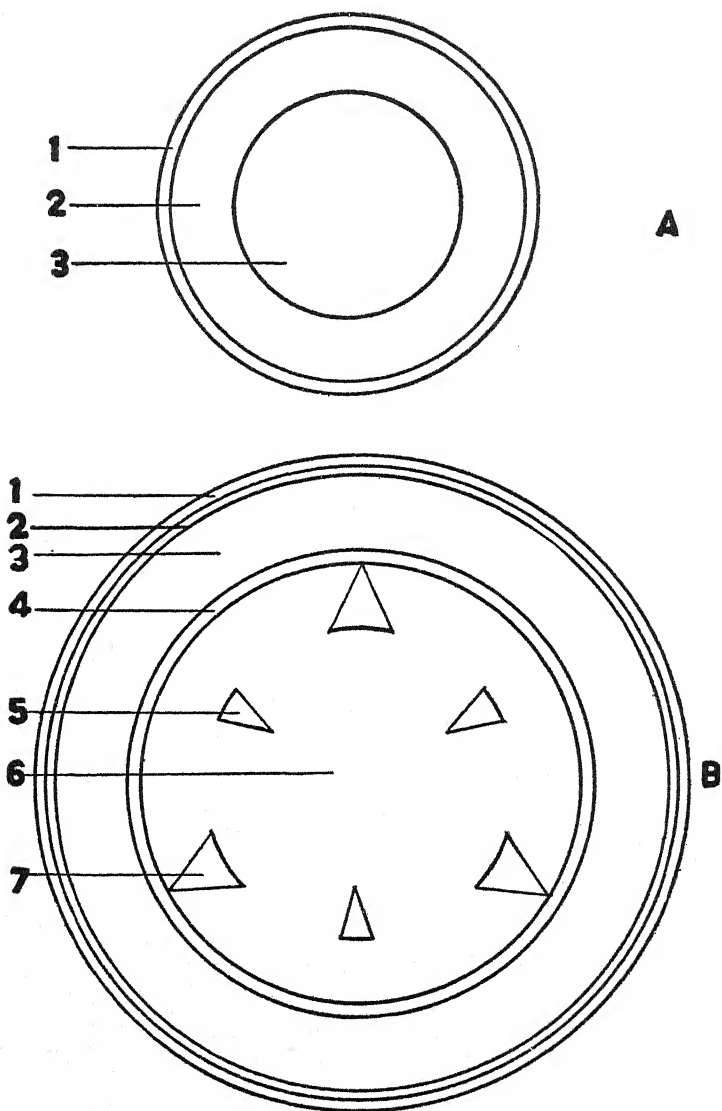
PLATE 29.—*A*. Arrangement of the Fundamental Tissue Layers in a
Root and Stem.

1. Dermatogen zone.   2. Periblem zone.   3. Plerom zone.

B. Arrangement of the Primary Tissues in the Root.

1. Epidermis.   2. Hypodermis.   3. Primary   Cortex.   4. Endodermis.
5. Xylem bundle.   6. Pith parenchyma.   7. Phloem bundle.

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region from which all other plant tissues are derived. Each of these zones (Plate 29), contains meristematic cells which soon produce primary tissues showing great differences in structure and contributing to the rapid development of the young plant.

Primary Root Tissues.—Differentiation of the
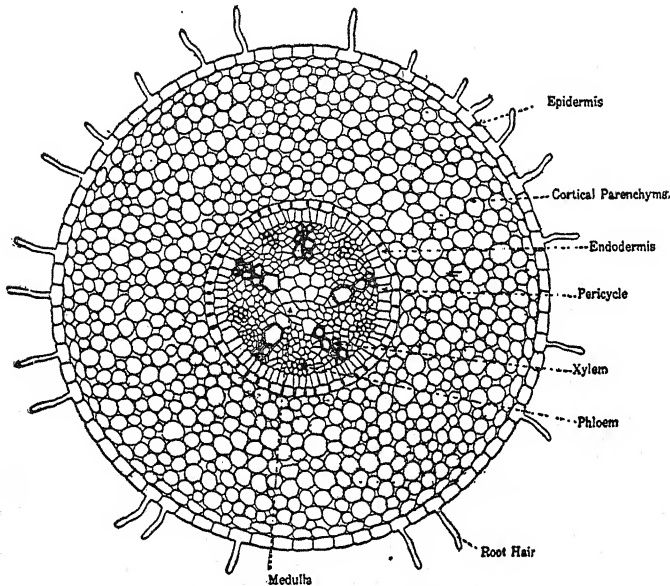
PLATE 30.—Cross-section (Diagrammatic) of a Young Dicotyledonous Root through the Root-hair Zone.
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fundamental tissue of the dermatogen, periblem and plerom zones results from changes in the character of the cell walls and from the activity of meristematic regions. In the dermatogen zone of the root, three distinct primary tissues are usually apparent. (Plates
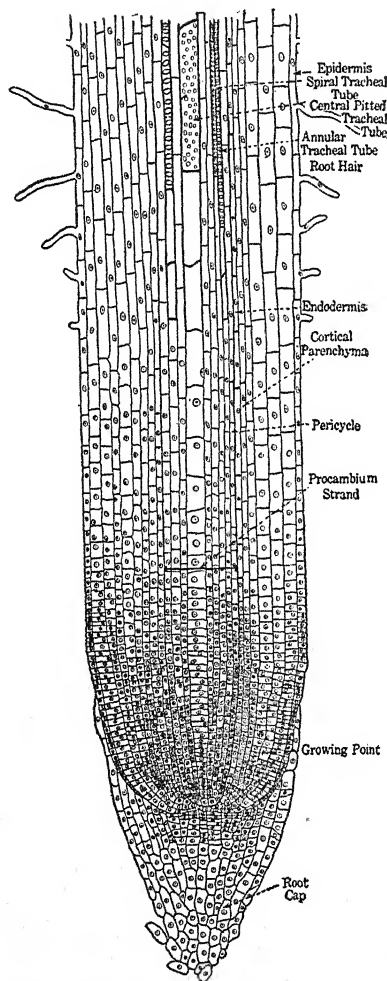
PLATE 31.—Longitudinal Section of the Root of Barley (*Hordeum Sativum*). Just back of the growing point is the region of elongation, which extends to the root-hair zone.

30, 31.) The outer layers of cells at the tip of the root form the root cap (Plate 31) and are r a t h e r thick-walled. This root cap serves to protect the more delicate structures from injury through contact with sharp particles of soil. The epidermal cells above the root cap give rise to root hairs (Plates 30, 31, 49) which are of importance in the absorption of materials from the soil. The *root hairs* are modified epidermal cells and appear as thin-walled projections from the latter. Portions of the root above those clothed with root hairs are covered with rather thick-walled epidermal cells termed the *primary epidermis*. In the periblem zone of the root, t h r e e primary tissues are present. The layer or layers of cells adjacent to

the primary epidermis constitute the *hypodermis*. The hypodermal cells are usually angled and thick-walled. The layer of cells adjacent to the plerom zone is termed the *endodermis*. Endodermal cells are usually thick-walled and bear a close resemblance to those of the hypodermal layer. The several layers of cells between the hypodermis and the endodermis are termed the primary cortex or *cortical parenchyma*. Cortical parenchyma cells represent the original periblem tissue and show little if any differences in structure from the latter. The most striking changes in character of cell wall due to development occur in the plerom region. Elongation occurs in certain groups of cells in this region and they acquire thickened walls through deposition of lignin, thus forming the *prosenchyma* or fibrous elements which give strength to the plant. Conducting elements or *ducts* are developed in the midst of these lignified cells. Each group of lignified cells, together with the associated ducts, is termed a *xylem bundle*, and these bundles may be arranged in a definite order in the plerom region (Plates 30, 31). Lignification of cell walls also occurs in groups of plerom cells other than those of the xylem bundles; but the conducting elements developed in these other lignified groups differ from the ducts in that they are not continuous. They are termed *sieve tubes*, because communication between the various cells is through perforated end walls. Each group of sieve cells, with its associated lignified tissue, is termed a *phloem bundle*, and these, like the xylem bundles, may be arranged in a definite order. The lignified supporting cells of a xylem bundle are termed *wood fibers*, while those in the phloem bundles

are termed *bast fibers.* As each xylem or phlœm bundle consists of a supporting or fibrous element and a vascular or conducting element, the term *fibro-vascular bundle* is applied to the combination. The xylem and phloem bundles are placed in a circle near the outer boundary of the plerom region, and in the first stages of development they usually alternate with each other. Narrow strips of unchanged plerom parenchyma extending between the fibro-
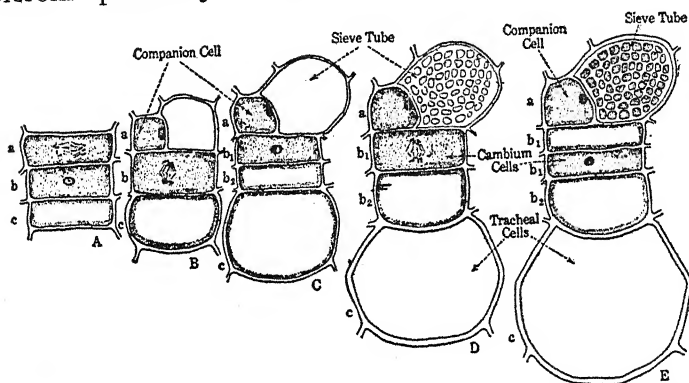
PLATE 32.—Stages Showing the Differentiation of Cambial Cells. Diagrammatic.

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vascular bundles form the *primary medullary rays.* The unmodified parenchyma in the center of the plerom is the pith. The arrangement of the various primary root structures is diagrammatically represented in Plate 29.

Secondary Root Tissues.—In certain orders of plants the primary tissues persist with but minor changes throughout life. In the higher orders many changes occur in these primary tissues, resulting in

the formation of secondary or permanent tissues, such as those of the bark, and an extension of the primary plerom elements. The primary root epidermis is replaced by bark structures which originate in the periblem zone. Certain of the primary cortical cells become meristematic and constitute the bark cambium or *phellogen*. The phellogen cells rapidly subdivide and the new tissue formed on their outer side forms the *periderm* or *bork*, while the tissues produced on their inner surface form the *phelloderm* or true *bark*. The phellogen retains its meristematic power throughout the life of the individual and can generally provide tissues to keep pace with internal growth. If, however, the internal growth is more rapid than the growth of the covering layers, rupture of the latter occurs. In this event a secondary phellogen layer is formed in the cortex, well interior to the break, and through its meristematic power forms cork to close the rupture. As a result of the secondary phellogen being deeply located in the cortex and by reason of the cork formation on its outer face, all external tissues are cut off from their sources of nutrition, and death ensues. These dead tissues, in combination with cork formed by the phellogen, constitute the composite covering tissue known as *secondary periderm*. The primary fibro-vascular bundles consist of either xylem or phloem, and, in the change to secondary structure, a meristematic tissue termed *cambium* is developed in connection with these. The cambium occurs on the outer face of each xylem bundle and on the inner face of each phloem bundle. The cambium arc on each xylem bundle produces xylem on its inner face and phloem elements on its
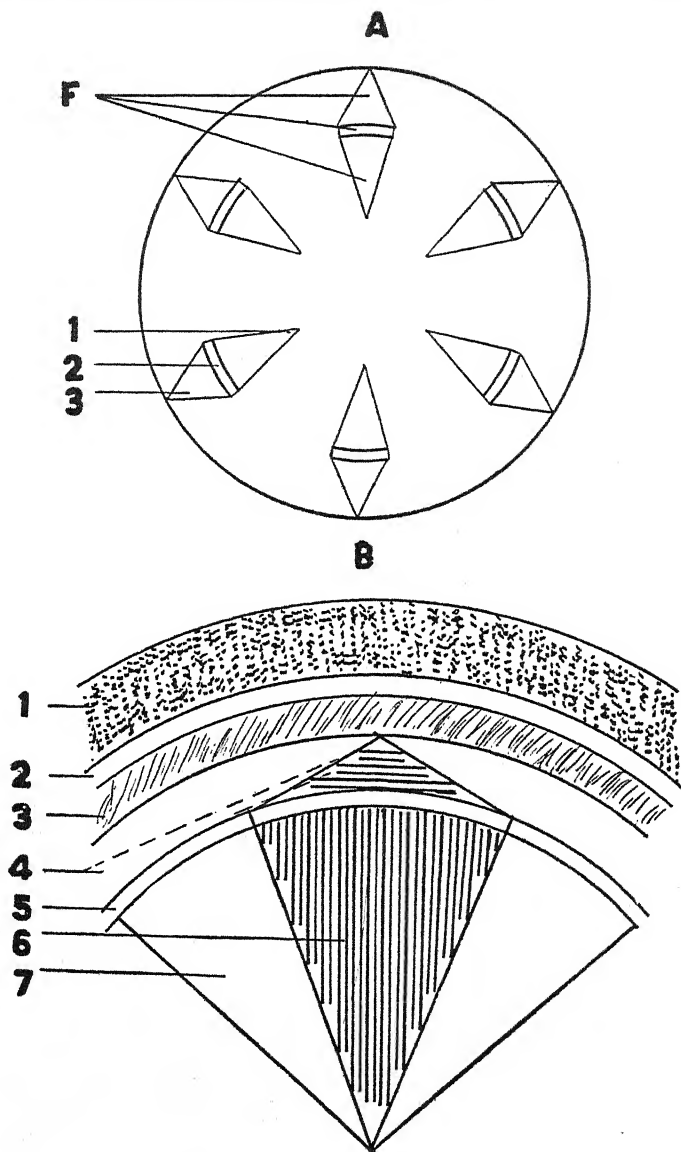
PLATE 33.—*A*. Completion of Fibrovascular Bundles.

F. Completed fibrovascular bundle.   1. Xylem elements.   2. Cambium.
3. Phloem elements.

B. Arrangement of Secondary Tissues in Roots and Stems.

1. Periderm (bork).   2. Phellogen. . 3. Phelloderm (bark).   4. Phloem ele-
ments. 5. Cambium. 6. Xylem elements. 7. Medullary rays.

outer side. Similarly the cambium arc on each phloem bundle produces xylem on the inner side and phloem upon the outer. Each fibro-vascular bundle now consists of xylem and phloem elements separated from each other by a strip of cambium. The bundles of secondary structure which have been completed by the cambium are termed *complete fibro-vascular bundles* in contradistinction to the *incomplete fibro-vascular bundles* of primary structure. Growth through activity of the cambium is continuous, and finally the parenchymatic tissues of the plerom zone in mature roots are entirely replaced by xylem (Plate 33). Formation of new fibro-vascular bundles takes place in the broad primary medullary rays, resulting in multiplication of these structures, which are derived from the original plerom parenchyma. The arrangement of the various secondary root structures is diagrammatically represented in Plate 33.

Primary Stem Tissues.—In sections of young stems the dermatogen, periblem and plerom zones are apparent. The dermatogen zone in the stem gives rise to an epidermis consisting either of one layer or of several layers which may show slight variations in structure. Tissues corresponding to those of the root cap and root hairs are not developed, although the stem epidermis may be clothed with various types of hairs. The primary stem epidermis may possess stomata or breathing pores, structures which are never present in the primary epidermal layer of roots. The periblem zone in stems gives rise to hypodermal and endodermal tissues similar to those of the root. The hypodermal cells may contain chlorophyl or green coloring material, which is never
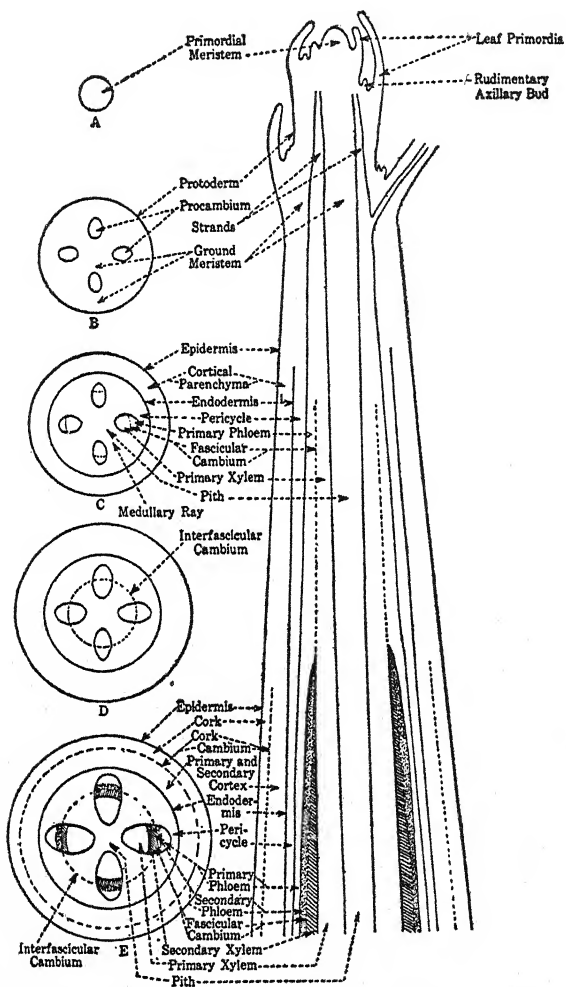
PLATE 34.—Diagram of Longitudinal and Cross-sections of a Typical Dicotyledonous Stem, Showing Primary and Secondary Growth.
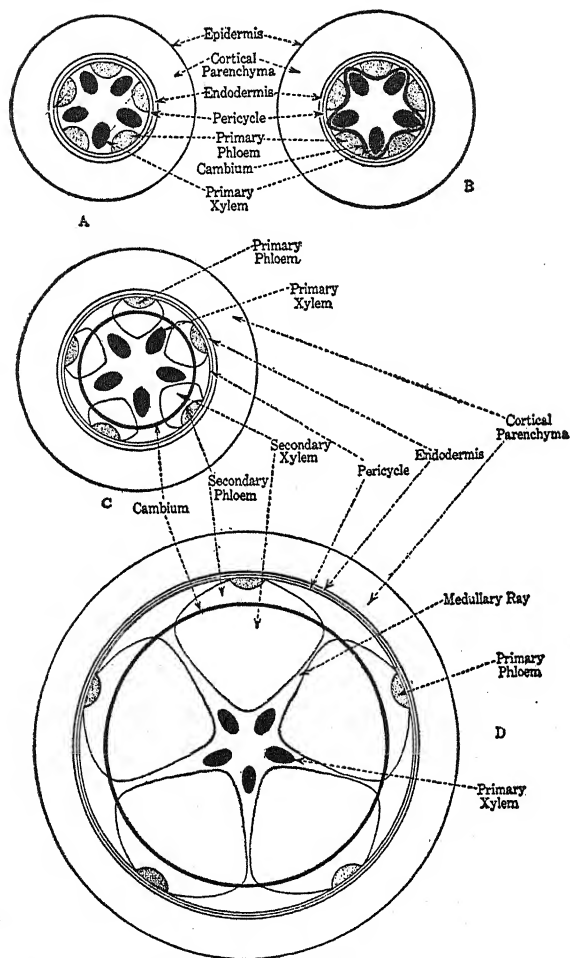
PLATE 35.—Diagrams Showing Stages in the Secondary Increase in Thickness of a Root. *A*, before the appearance of cambium. *B*, the formation of the cambium ring. *C* and *D*, stages in the development and growth of secondary phloem and xylem. Secondary increase in thickness due to the activity of the phellogen is not shown.

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present in the corresponding tissue of roots.   In most
instances, the endodermal cells of primary stems are
not as well developéd as those of roots.   The plerom
zone of primary stems is quite different from that of
primary roots, as regards arrangement and develop-
ment of tissues.   The fibro-vascular bundles developed
in the plerom region of the primary stem are *complete*
bundles, showing phloem, xylem and cambium elements
even in the earlier stages of growth (Plate 33).   There-
fore the primary fibro-vascular bundles of the stem
show an arrangement found only in the secondary
bundles of the root.

   Secondary Stem Tissues.—In annual plants the
primary stem structures heretofore described persist
throughout the short life of the individual; but in
perennials, provision must be made for a more dur-
able covering tissue and the extension of the plerom
structures.   The primary epidermis is replaced by
periderm tissues produced by a phellogen developed
in the primary cortex.   The periderm of stems, like
that of roots, is often cast off or ruptured because of
expansion of the inner tissues through growth.   *Pri-
mary periderm* thus destroyed is replaced by a *sec-
ondary periderm* arising from the secondary phellogen
layers, as noted in the section on secondary root
tissues.   The hypodermal and endodermal layers dis-
appear with the formation of phellogen within the
primary cortex.   The primary fibro-vascular bundles
increase in size through the addition of xylem and
phloem elements by the cambium.   The short arcs of
cambium between the xylem and phloem portions of the
complete fibro-vascular bundles are extended laterally
so as to form a complete cambium ring or circle.

New fibro-vascular bundles are formed in the broad medullary rays which extend between the original bundles. Although new woody elements are continuously added to the original xylem bundles, the growth of wood never entirely replaces the original plerom tissue in the center of the stem. This unchanged plerom parenchyma in the center of stems is termed the *pith*. In roots, the woody tissues entirely replace the plerom parenchyma; therefore, the presence of a pith region or a space representing it is characteristic of stem structure.

Although the primary tissues serve well enough in the earlier stages of growth, full development necessitates the production of secondary or permanent tissues, and these may be roughly divided into two groups, *parenchyma* and *prosenchyma*. Representative parenchyma cells are found in the undifferentiated cellular structures of the three zones in the embryo. They are characterized by thin walls and the possession of protoplasmic contents. Typical prosenchyma cells are formed in the plerom region of the embryo. They differ from parenchyma in the possession of thick walls, and the protoplasmic contents are inconspicuous or lacking. These distinctions are not absolute, and the form or shape of the cell may be used as a basis for further classification in doubtful instances. Prosenchymatic elements are usually in the form of long fiber cells with sharp-pointed ends, whereas parenchymatic cells are usually spherical or isodiametric in shape and do not possess pointed ends.

SUMMARY OF ROOT AND STEM TISSUES

ROOT

Fundamental Tissues.	Primary Tissues.	Secondary Tissues.
Dermatogen....	{ Root cap, Root hairs, Primary epidermis }	All replaced by structures derived from phellogen
Periblem....	{ Hypodermis, Primary cortex, Endodermis }	Phellogen { Bork (Periderm), Bark (Phelloderm)
Plerom....	{ Fibro-vascular bundles (incomplete), Medullary rays, Pith parenchyma }	Fibro-vascular bundles (complete), Secondary medullary rays, Replaced by woody tissues

STEM

Fundamental Tissues.	Primary Tissues.	Secondary Tissues.
Dermatogen	Primary epidermis	Replaced by structures arising from phellogen
Periblem....	{ Hypodermis, Primary cortex, Endodermis }	Phellogen { Bork (Periderm), Bark (Phelloderm)
Plerom....	{ Fibro-vascular bundles (complete), Medullary rays, Pith parenchyma }	Secondary fibro-vascular bundles (complete), Secondary medullary rays, Permanent pith

CHAPTER VII

THE COVERING TISSUES

Covering, or protective tissues, are found on all exposed surfaces of the plant. The general function of these tissues is the protection of the vital parts of the plant from injury due to climatic variation or other agents beyond the control of the plant. In northern or even temperate latitudes the plant is subjected to great and fairly rapid fluctuations in temperature, which, if not guarded against, would cause injury to the more delicate plant organs. The covering tissues act as insulators and prevent temperature variations from injuring the plant. In certain instances the plant possesses the faculty of adjusting itself to long-continued periods of high or low temperature; but this adjustment entails a modification of the covering tissues. Plants in tropical regions require protection against the excessive heat of the sun during the dry season and an undue access of water during the rainy period. Both of these adverse conditions are met by modifications in the structure of the covering tissues. While these covering tissues are always located on the exposed surfaces of the plant, it must not be taken for granted that all tissues so located function as covering structures. The root hairs, while superficial and arising from an epidermal layer, must be placed with the tissues for absorption

rather than with the covering tissues, because of their function, and the same holds good for stomata and the various modifications of epidermis which possess secretory functions. By reason of differences in location, formation, structure, and chemical composition, the true covering tissues of the plant are subdivided into; (a) epidermal tissues, including plant hairs, and (b) periderm or cork.

EPIDERMAL TISSUES

Epidermal tissues occur on the exposed surfaces of all plant organs and parts other than those of woody structure. Thus young roots, leaves, green stems, fruits, seeds and floral parts possess this covering tissue classed as epidermis. The epidermal tissues are derived from the outermost layer of the dermatogen region, and the cell walls are more or less thickened by deposition of cutin, in or around the original cellulose wall. Cutinized cell walls are practically waterproof. Silicates and other inorganic materials are often present in the epidermal tissues of grasses, and probably aid in the support of those plants in which poor development of fibrous supporting tissues is noteworthy. Cutinized tissues frequently have the power of secreting or producing waxes and resins. The gloss on the surfaces of certain leaves is due to the presence of a waxy material which serves as an additional protection against access of water through the epidermis. Resins and sticky substances, occasionally present on the surfaces of leaves and stems, serve the purpose of preventing insect attacks and keeping ants or other crawling insects from gaining access
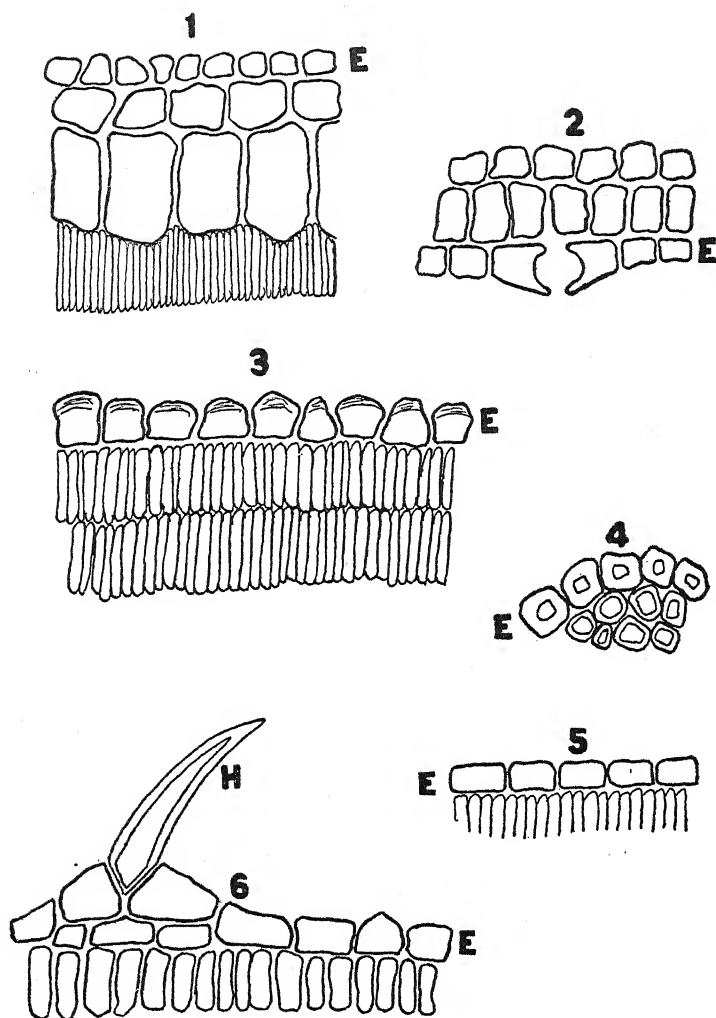
PLATE 36.—Sectional Views of Leaf Epidermis.

1. Upper epidermis, Ficus leaf. 2. Lower epidermis, Ficus leaf. 3. Upper epidermis, Eucalyptus leaf. 4. Epidermis of Pine leaf. 5. Upper epidermis, Orange leaf. 6. Upper epidermis, Geranium (Pelargonium) leaf, E = epidermis. H = hair.

to the flowers. Many of the resins are strong anti-septics and will prevent infection of plant tissues.

Membranous Epidermal Tissues.—The epidermal tissue covering young roots, leaves, green stems, floral organs and several classes of fruits is in the form of a thin layer of cells. The epidermal membrane of leaves and green stems is colorless and transparent, while that covering roots, fruits and floral parts is usually colored by deposition of various cell contents and is therefore less transparent. The epidermal membrane of leaves (Plate 36) and other aerial plant parts is seldom more than one layer of cells in thickness; and the individual cells are set so closely together that air and water cannot gain access to the inner tissues except through specialized organs called *stomata* (singular, *stoma*). Stomata are distributed among the epidermal cells, especially those of the lower leaf surface. Each stoma consists of two semicircular cells surrounding an oval opening or breathing pore. Stomata are of importance in the respiratory processes of the plant and cannot be classed as covering tissues. Certain leaves, on sectional view, show several layers of cells apparently similar in structure to the epidermal cells to which they are attached. These layers are termed *sub-epidermal* cells and occur frequently in leaves of tropical plants, where they serve as a protection against the excessive heat to which such plants are exposed. The epidermal membrane of the young root may be more than one layer of cells in thickness, and certain of the cells give rise to root hairs. While the root epidermis is a true covering tissue, the root hairs are absorbing structures and will be considered in a separate section. The
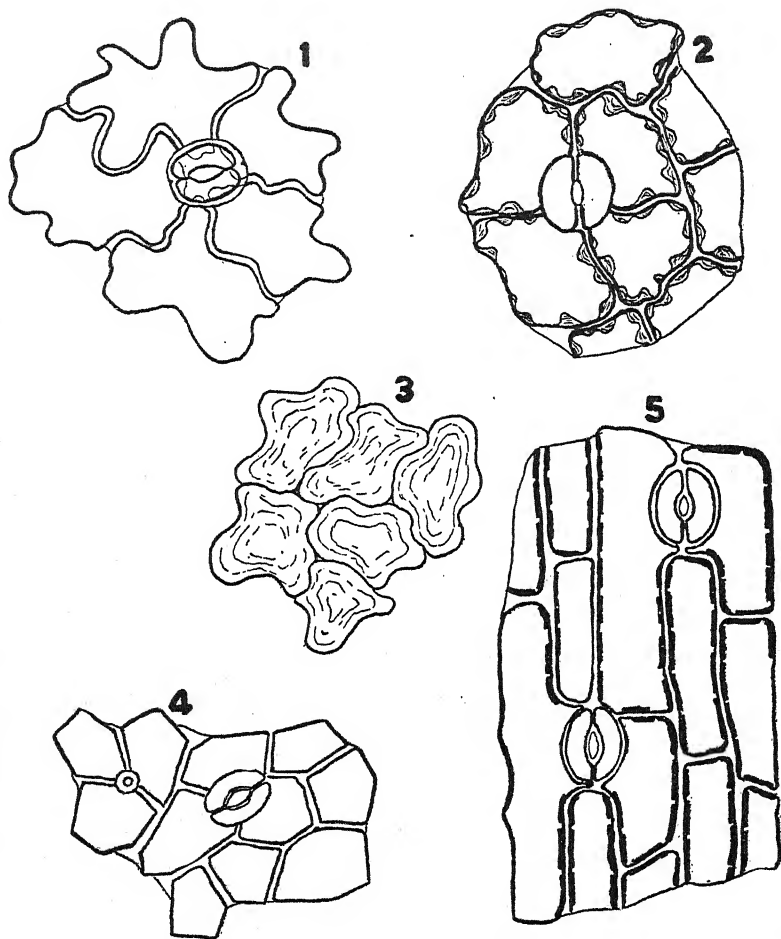
PLATE 37.—Surface Views of Leaf Epidermis.
1. Hepatica leaf (wavy walls). 2. Chimaphilla leaf (beaded walls). 3. Henbane leaf (wavy and striated walls). 4. Senna leaf (angled cells). 5. Convallaria leaf (beaded walls).

individual epidermal cells, as seen on surface view (Plate 37) are polygonal in shape, or else show a very irregular outline, because of their wavy walls. Sectional views show that the upper or exposed surface of the cell is thicker-walled than the surfaces in contact with other cells. The cell walls frequently show markings which are of use in identification. *Striations* are markings or lines parallel with the wall of the cell as seen on surface view. These markings may possibly be due to the deposition of cutin or other protective substances in successive layers. The thick-ening of the exposed wall may be partially extended into the side walls of epidermal cells, thus affording greater protection. *Beaded cell walls* are side walls which have become thickened at irregular intervals around their circumference or margin. Roughening on the exposed surfaces of epidermal cells may be due to striations, beading or presence of plant hairs.

Thickened Epidermal Tissues.—The epidermal tissue covering seeds and certain classes of fruits is in the form of one or more layers of thick-walled cells, constituting the testa or outer coat of seeds and the exocarp of fruits (Plate 38). Stomata are never present in seed epidermis but are occasionally present in fruit epidermis. Protection of the embryo and of the nourishing materials in the seed is the chief function of the testa. The seed epidermis is necessarily thicker and stronger than the membranous epidermal tissues, because the seed must retain its vitality for long periods even under the most adverse conditions. The epidermal cells of seeds and fruits usually contain pigment materials and therefore appear colored. The subepidermal layers may secrete
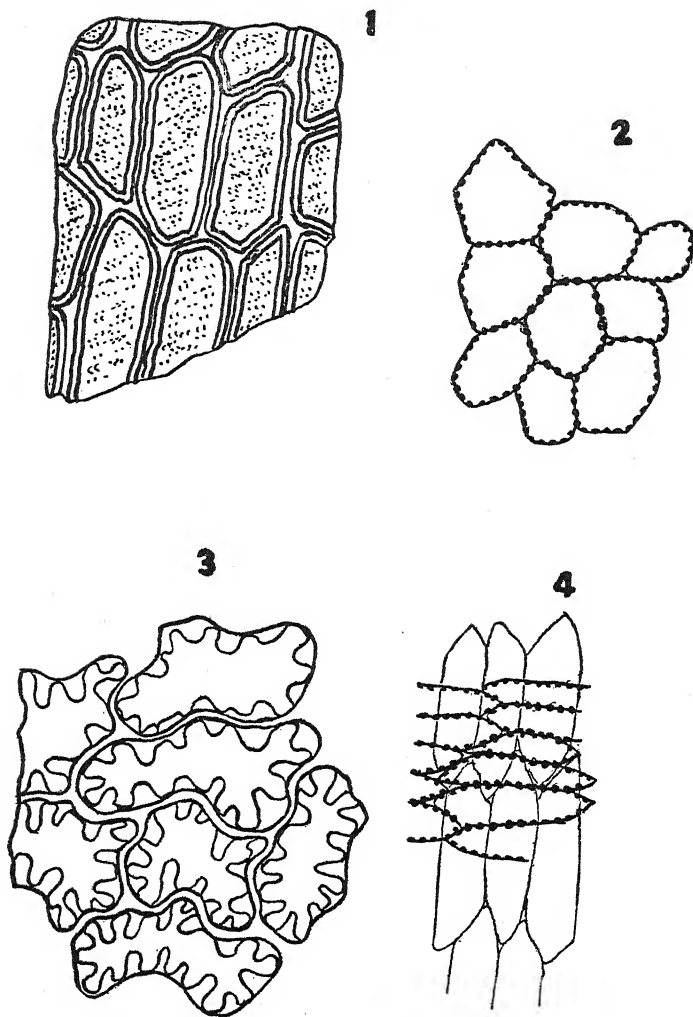
PLATE 38.—Surface Views of Seed Epidermis.

1. Lobelia seed (thick walled, angled cells). 2. Mustard seed (finely beaded walls). 3. Capsicum seed (wavy and thick beaded walls). 4. Wheat seed (angled and finely beaded walls).

mucilaginous substances through modifications of the cell walls. The swelling which occurs when the mucilaginous substance comes into contact with water tends to rupture the tough epidermis, thus making possible the egress of the embryo. Waxy secretions are occasionally present on the surface of the seed.

PLANT HAIRS

Plant hairs or *trichomes* are prolongations or outgrowths of the epidermal cells of aerial portions of the plant. In certain instances the trichome is merely an expansion of the exposed surface of the epidermal cell, while in others, cell division has occurred and the hair consists of several well-defined cells. Trichomes may occur on the epidermal surfaces of leaves, green stems, floral organs, fruits, and occasionally seeds. The cells are cutinized and, in certain hairs, secretory organs or glands are present. The absence or presence of glands is the primary basis of classification into *non-glandular* and *glandular* hairs, but the secretory function of the latter type places them as structures for synthesis rather than true covering elements. The cells of a trichome may be living or dead. Living hairs are characterized by the possession of protoplasmic contents and inclusions within the cell. In dead hairs the cell cavities are filled with air. Although cutinized cell walls are the usual rule, there are instances where the walls of hairs consist partly of lignified tissue or even of inorganic materials; trichomes of this sort are likely to be thicker and more like bristles.

Non-glandular Trichomes.—The functions of nonglandular hairs include protection against temperature

changes, excessive evaporation of water, intense illumination, and attacks of insects. The simplest type of non-glandular hair consists of a slight projection of the exposed epidermal surface. This form of hair is termed a *papilla* (plural, *papillæ*) and appears as a wart or blister upon the epidermal surface (Plate 39). On surface view papillæ appear as thick-walled circles, one within each epidermal cell. On sectional view they appear as a series of elevations on strips of epidermal tissue. There is a possibility that papillæ function to intercept light rays and reflect them into the interior cells of the leaf.

Classification.—Non-glandular hairs may be classified according to the number of cells in the hair, the number of hairs in the group, the presence or absence of branches, and the arrangement of the cells in each hair. This classification may be tabulated as follows:

> (*a*) Number of cells in the hair;
>   unicellular—multicellular.
> (*b*) Number of hairs in the group;
>   simple—compound or aggregated.
> (*c*) Branching;
>   non-branched—branched.
> (*d*) Series of cells in the hair;
>   uniseriate—multiseriate.

Unicellular hairs consist of but one cell, whereas *multicellular hairs* consist of many cells. If the hairs occur individually they are termed *simple* or *solitary*, in distinction to *compound* or *aggregated hairs* which are grouped and usually united at the basal portion. While the individual parts of a compound hair may be unicellular or multicellular, the aggregate is always multicellular. The terms branched and non-branched
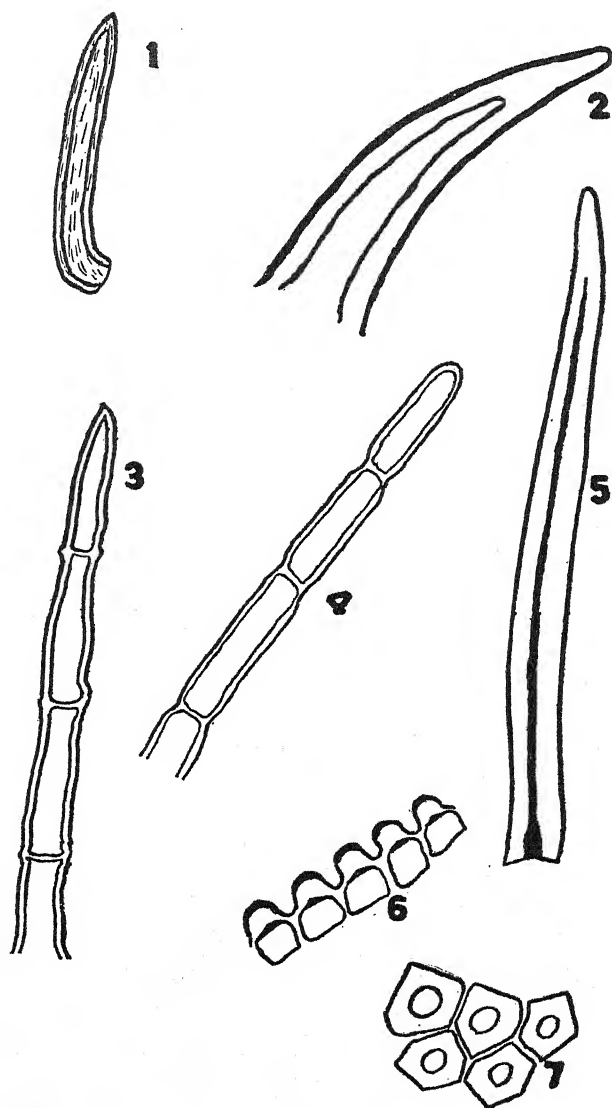
PLATE 39.—Nonglandular Plant Hairs.

1. Senna leaf. (Simple, unicellular, nonbranched.) 2. Cannabis herb. (Simple, unicellular, nonbranched.) 3. Matico leaf. (Simple, multicellular, nonbranched.) 4. Digitalis leaf. (Simple, multicellular, nonbranched.) 5. Tea leaf. (Simple, unicellular, nonbranched.) 6. Coca leaf, Papillæ, sectional view. 7. Coca leaf, Papillæ, surface view.
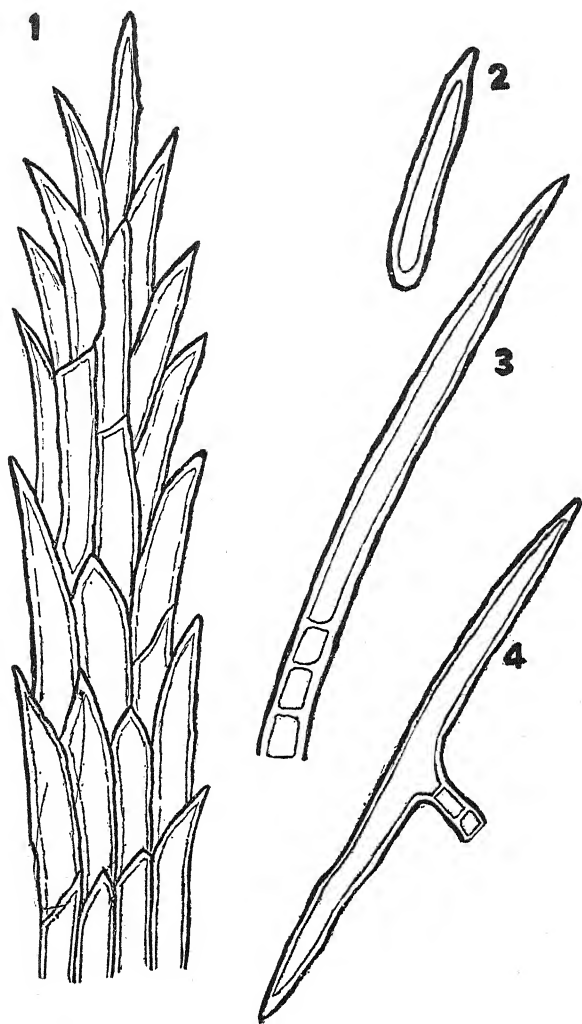
PLATE 40.—Nonglandular Plant Hairs.

1. Arnica flowers. (Simple, multicellular, multiseriate.) 2. Coca leaf stem. (Simple, unicellular.) 3. Anthemis flowers. (Simple, multicellular, uniseriate.) 4. Absinthium leaves. (Simple, multicellular.)

are self-explanatory; but the distinction between compound and branched trichomes must be kept clearly in mind. Branched forms differ from compound forms in that the former project from the epidermal cell as a single hair, and the branching occurs at some point above the origin. *Uniseriate trichomes* are multicellular forms in which the several cells composing the hair are arranged in a single row or series. *Multiseriate trichomes* are likewise multicellular, but the cells composing the hair are arranged in several parallel rows or series. In describing plant hairs the four points enumerated above must be taken into consideration. The hairs of cannabis and senna are unicellular, simple and non-branched. The hairs of digitalis and matico are multicellular, simple, non-branched and uniseriate. The hairs of althæa leaves are multicellular, compound, with unicellular parts and non-branched. Among the several kinds of hairs present in arnica flowers, we find multicellular, simple, non-branched and multiseriate types. Mullein leaf possesses simple, multicellular, branched, uniseriate trichomes. The various types of trichomes are illustrated in Plates 39, 40 and 41.

The cells of non-glandular trichomes often show markings or other peculiarities which may be of importance in identification work. Striations extending parallel with the length of the hair are often present. The *papillose surface* of peppermint and stramonium hairs is caused by small projections upon the surface of the hair. In absinthium the large *terminal* cell of the hair stands at right angles to the *basal cells*, a characteristic observed in but a small number of plants. The hair of anthemis shows a terminal cell
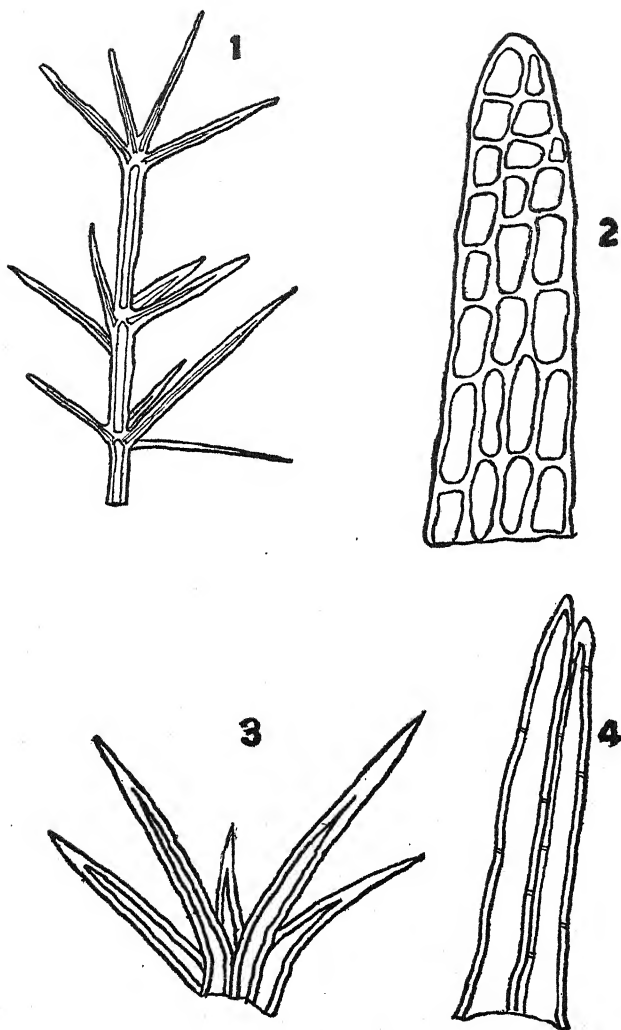
PLATE 41.—Nonglandular Plant Hairs.

1. Mullein leaves. (Simple, multicellular, branched.) 2. Geranium (Pelargonium) stem. (Multicellular, multiseriate.) 3. Althæa leaves. (Compound, with unicellular divisions.) 4. Arnica flowers. (Bicellular, biseriate.)

many times longer than the basal cells. One type of hair occurring in thyme leaves is two-celled with the terminal cell bent at a sharp angle to the basal cell. *Biseriate* or twin hairs are found in arnica flowers. Hairs frequently show projections or barbs along the edge of the cell. In multicellular hairs these projections may be formed by individual cells, but in unicellular hairs they are *emergences* or projections from the cell wall. Barbed hairs play an important part in the protection and distribution of seeds. The hairs of stinging nettle (Urtica dioica) contain secretions which, if injected under the skin, cause severe irritation. In other instances (particularly cowage, *Mucuna pruriens*), plant hairs are provided with recurved barbs by means of which they become firmly embedded in the skin and thus act as irritants.

Glandular Trichomes.—A glandular trichome consists essentially of a gland or secreting organ which is in direct contact with the epidermal surface, or is raised above this surface by means of a stem or stalk. *Sessile glandular hairs* are directly attached to the epidermis. In *stalked glandular hairs* the gland is raised above the epidermal surface and attached to it by the stalk. The gland or secreting organ may be unicellular or multicellular. The terms used in the description of non-glandular hairs are used in reference to the stalk of a glandular hair. The glandular hairs of cannabis possess a multicellular gland with multiseriate stalk. The glandular hairs of pelargonium (commonly called geranium) possess a unicellular gland upon a multicellular, uniseriate stalk. Various types of glandular hairs are illustrated in Plate 42. The formation of glandular hairs and
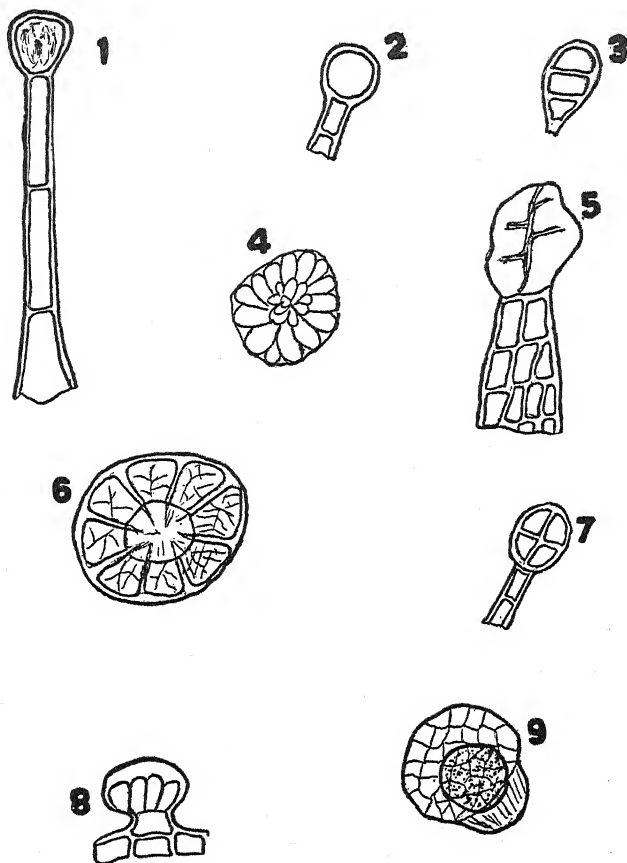
PLATE 42.—Glandular Plant Hairs.

1. Geranium (pelargonium) stem. (Multicellular stalk, unicellular gland), 2. Sage leaves. (Multicellular stalk, unicellular gland.) 3. Sumac fruit. (Sessile. multicellular gland.) 4. Kamala fruit. (Sessile, multicellular gland in surface view.) 5. Cannabis herb. (Multiseriate stalk, multicellular gland.) 6. Peppermint leaves. (Sessile, multicellular gland in surface view.) 7. Stramonium leaves. (Multicellular stalk, multicellular gland.) 8. Lavender flowers. (Sessile, multicellular gland in sectional view.) 9. Lupulin. (Sessile, multicellular gland in sectional view.)

the products secreted will be considered under the
head of Secreting Tissues.

<div align="center">PERIDERM</div>

Periderm occurs on the exposed surfaces of woody
roots and stems. The primary epidermal tissues
originating from the dermatogen zone do not afford
sufficient protection to mature parts of the plant, nor
can they keep pace with the growth of the inner tissues.
The necessity for a stronger covering arises, and is
met by the production of cork by a meristematic tissue
located in the primary cortex or immediately beneath
the primary epidermis. This meristematic tissue is
termed *phellogen* or *bark cambium*, and produces cork
tissue on its outer surface and phelloderm upon its
inner face. With the exception of a region near the
phellogen, the cork layers are composed of dead cells.
The phellogen tissues are of importance in bark struc-
ture and will be considered under that heading in a
subsequent chapter. The walls of cork cells are com-
posed largely of suberin and the cell contents usually
include tannins. The individual cells are always dark-
colored, and are so fitted together that very little
intercellular space is apparent. Upon surface view
the cells are usually polygonal in outline and occur
in thick masses in which the outlines of the individual
cells are obscure. Upon sectional view the cells appear
rectangular or oblong in form and are usually arranged
in regular rows (Plate 43). Irregular thickening of
the walls of cork cells may be noted in some instances,
the walls toward exposed surfaces being usually so
thickened. In fully mature plants, especially old
trees, the periderm consists of cork together with
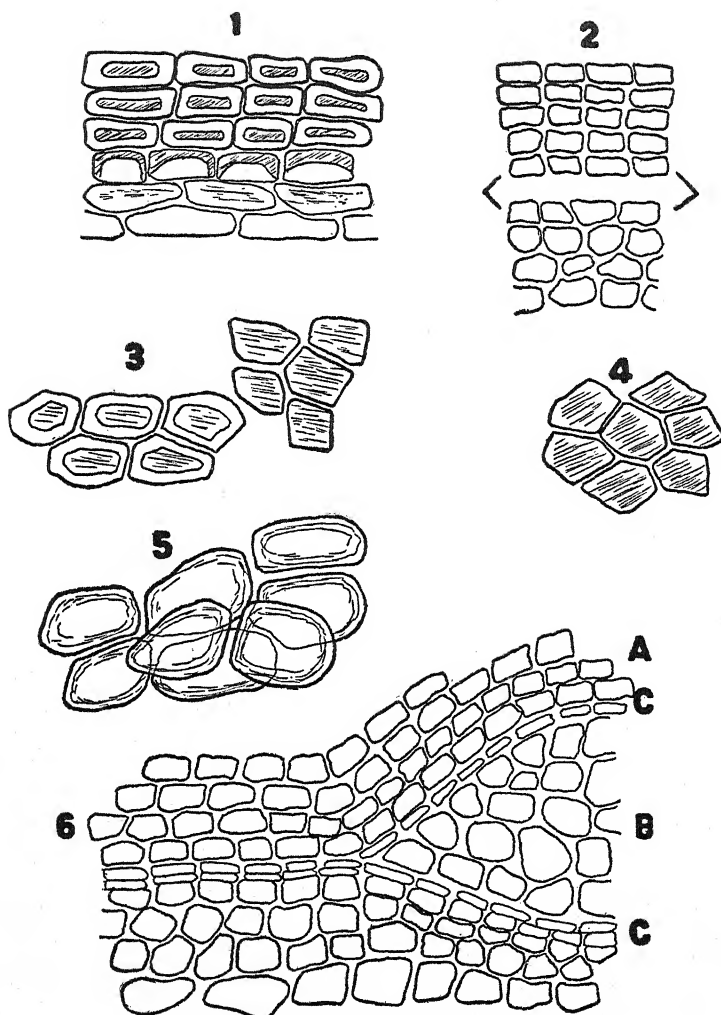
PLATE 43.—Cork Tissue.

1. Saigon Cinnamon. (Sectional view.) 2. Cascara Sagrada. (Sectional view.) 3. Saigon Cinnamon. (Surface view.) 4. Cascara Sagrada. (Surface view.) 5. Viburnum Opulus. (Surface view.) 6. Euonymus, Stem bark. (Sectional view.) Formation of secondary periderm. *A.* Primary periderm. *C.* Layers of phellogen. *B.* Parenchyma included in secondary periderm.

parenchyma, fibrous elements and in some instances, stone cells (Plate 43). These additional structures result from the formation of corky tissues beneath fissures or cracks in the original periderm. The corky layers are produced by a secondary phellogen region established well within the fissure. Increasing amounts of cork are produced and the tissues external to this new periderm are thus forced outward, finally becoming part of the outer bark or bork.

The functions of cork cells are to afford protection against mechanical injury, insect attacks and access of water to the inner tissues, and to prevent loss of water through excessive evaporation.

CHAPTER VIII

SUPPORTING TISSUES

THE necessity for a framework or skeleton arises fairly early in the life of the plant, and the supporting or mechanical tissues which serve this purpose are developed from groups of cells in the plerom zone. The process of formation involves the deposition of lignin around the original cellulose walls of certain plerom cells. Lignification of the cell walls may be accompanied by a great increase in the length of the plerom cells so changed. The mechanical tissues not only serve as a framework, but are also protective tissues, in that they enable the plant to resist the effects of wind, the attacks of animals and other external forces which might injure or uproot it. In large trees supporting cells form the bulk of the tissues present, because it is necessary that a weight of perhaps several hundred pounds be sustained in the position most favorable for growth.

The supporting elements of the plant include fibrous tissues, sclerenchymatic or stone cell tissue and collenchymatic tissue. While fibrous tissues and collenchyma may be formed early in plant life, stone cell tissue represents a later development. The endodermal cells which form the inner boundary of the periblem region possess thickened walls and may function as a supporting tissue in the early stages of growth.

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Fibrous Tissues.—Fibrous tissues are found in practically all parts of the plant excepting the seed, although the relative amount present in the different organs varies considerably. The greatest amount is found in woody stems and the least in floral organs. Each fiber is an elongated or spindle-shaped cell and is always several times longer than it is broad. The ends of these fiber cells may be sharp-pointed or blunt, with oblique end walls. Occasionally the walls retain part of the original cellulose around which the lignin has been deposited, and this combination is termed *lignocellulose*. The protoplasmic contents are usually lacking in fiber cells, and in well-matured plants the fibers are to all intents dead tissue. Very frequently the cavities of these dead fibers contain resins, tannins, and other protoplasmic contents which are serviceable in preventing decay. The fiber-cell walls are usually more or less thickened through the lignification process and in some instances appear *striated*, or show fine markings extending parallel to the length of the fiber. The cell cavity is termed the *lumen* of the fiber and may be of large or small size, depending upon the thickness of the wall. Certain fiber cells show pores or small openings in the wall of the cell through which materials may pass from the intercellular space into the lumen. In comparatively few instances the fibers are branched and, instead of appearing as long tapering cells, are irregular in form. Non-protoplasmic contents or cell inclusions occasionally occur in the lumen of a fiber, and may include calcium oxalate crystals, starch and coloring materials. Fibers containing calcium oxalate crystals within the lumen or apparently upon the surface of the fiber are termed *crystal-bearing*.
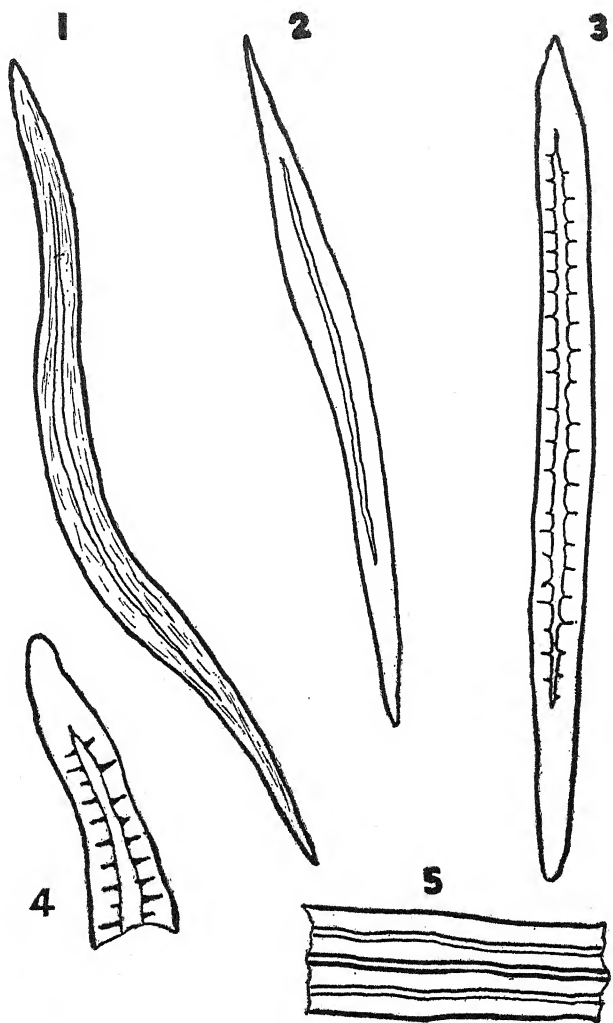
PLATE 44.—Fibers.

1. Baptisia root. (Nonporous, striated.) 2. Ceylon Cinnamon bark. (Nonporous, nonstriated.) 3. Hydrangea root. (Porous, nonstriated.) 4. Echinacea root. (Porous, nonstriated.) 5. Senna leaf, fragment. (Nonporous, nonstriated.)
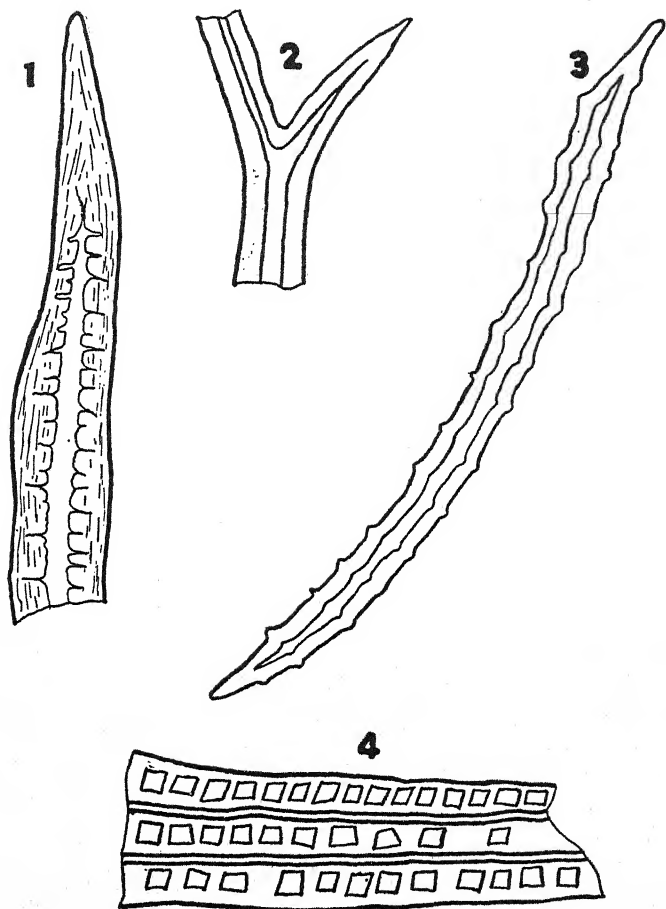
PLATE 45.—Fibers.

1. Cinchona bark. (Porous, striated.) 2. Tonga root, fragment. (Branched, nonporous, nonstriated.) 3. Nettle root. (Porous, nonstriated.) 4. Cascara Sagrada bark, fragment. (Crystal-bearing.)

In descriptions and illustrations of fiber cells the longitudinal or side view is usually considered, as on sectional view one merely obtains an idea of the diameter of the fiber and the relative size of the lumen. In sectional view, fibers are rarely circular in outline, as one or more sides are flattened because of the pressure of surrounding tissues. Various types of fibers are illustrated in Plates 44 and 45.

Classification.—In describing fibers, the following points should be noted:

1. Presence or absence of pores;
   porous and non-porous types.
2. Presence or absence of striations;
   striated and non-striated types.
3. Branching;
   branched and non-branched types.
4. Presence or absence of crystals;
   crystal-bearing and non-crystal-bearing types.

Wood and Bast Fibers.—In tracing the origin of plant tissues we learned that two types of conducting tissues were developed in the plerom region, and that each of these was associated with a fibrous or supporting tissue. One of these supporting tissues was termed *xylem fiber* or *wood fiber* and the other *phloem fiber* or *bast fiber*. There are slight histological differences between wood fibers and bast fibers. Wood fibers are usually thicker-walled and shorter than bast fibers. Pores, when present in wood fibers, are usually oblique and few in number, whereas in bast fibers the pores are horizontal to the long axis of the cell and rather numerous. As wood fibers are more brittle than bast fibers they are more apt to be broken in grinding, and while complete bast fiber cells may easily be found in
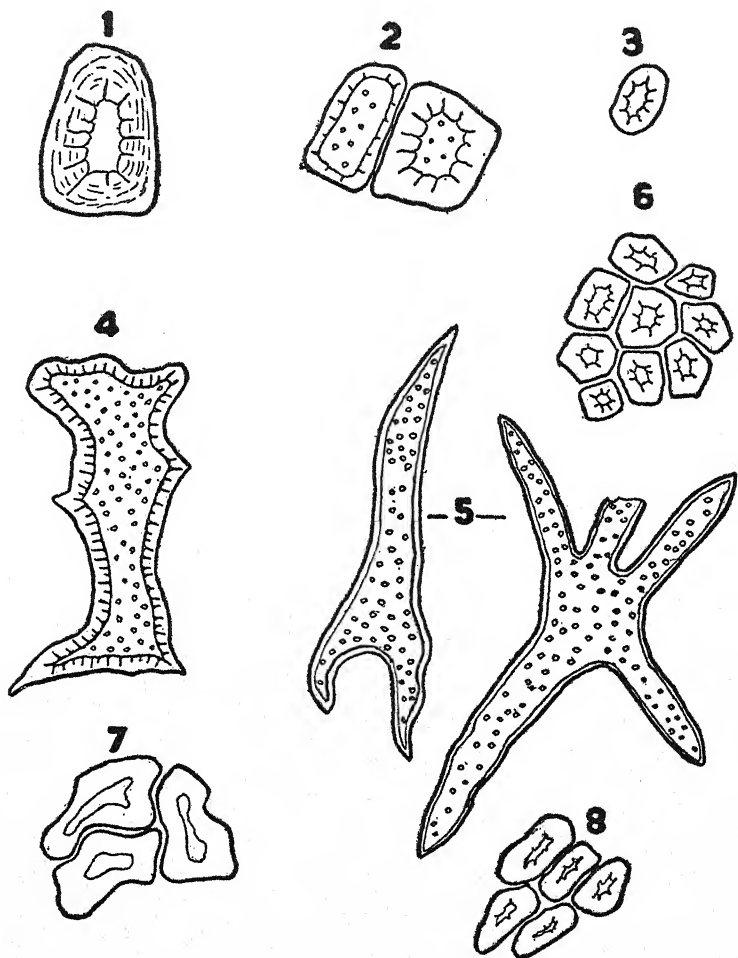
PLATE 46.—Stone Cells.

1. Chionanthus bark. (Porous, striated.) 2. Ceylon Cinnamon bark. (Porous, nonstriated.) 3. Xanthoxylum bark. (Porous, nonstriated.) 4. Tea leaf. (Branched, porous.) 5. Pond Lily rhizome. (Branched, porous.) 6. Pepper fruit. (Porous, nonstriated.) 7. Prunus bark. (Nonporous, nonstriated.) 8. Cascara bark. (Porous, nonstriated.)

powdered materials it is seldom that wood fiber cells remain intact.

Sclerenchymatic Tissue.—Sclerenchymatic tissues or *stone cells* may be found in all parts of the plant with the exception of the floral organs. The seed coat often contains large amounts of this tissue as it forms a hard protecting envelope for the embryo and other parts of the seed. Stone cells are particularly apt to be present in the stems of leaves, flowers and fruits as well as in barks. Stone cell walls are composed of ligno-cellulose. The deposition of lignin with the consequent formation of stone cells takes place at a later period than the transformation of plerom cells into fibrous tissue. The term sclerenchyma is sometimes indiscriminately applied to all hard tissues, and when so used includes wood fibers, bast fibers and stone cells. Stone cells appear as rectangular, polygonal and irregular thick-walled cells, the length seldom being more than three times the diameter. The thickness of the wall and, consequently, the size of the cell cavity vary considerably in the different types. Striations and pores may be apparent and the cavity may contain inclusions.

In describing stone cells the following points should be noted:

1. Presence or absence of pores;
   porous and nonporous types.
2. Presence or absence of striations;
   striated and nonstriated types.
3. Branching;
   branched and nonbranched types.

Stone cells, with the possible exception of the branched types, remain intact during grinding and
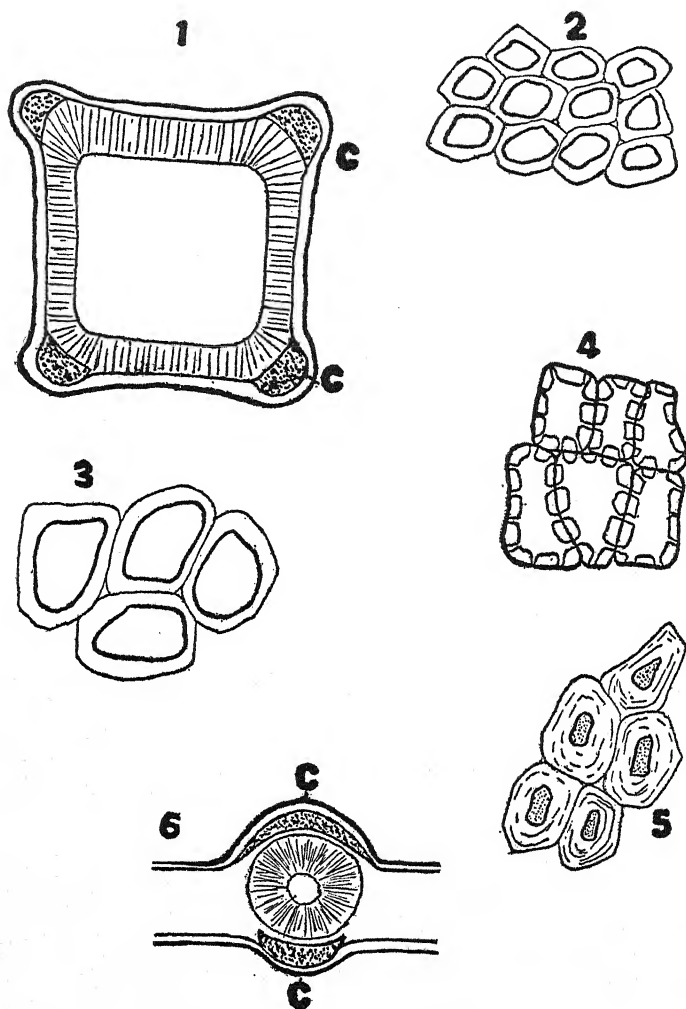
PLATE 47.—Collenchymatic Tissue.

1. Peppermint stem.   Arrangement of collenchymatic (*C*), tissues at angles of the stem. 2. Peppermint stem.   3. Sabal seed.   4. Colchicum seed.   (Porous type.)   5. Nux Vomica seed.   (Striated type.)   6. Arrangement of collenchymatic tissues around the midvein of a leaf.   *C* = collenchyma.

owing to their close contact, usually occur as masses in powdered materials. Various types of stone cells are illustrated in Plate 46.

Collenchymatic Tissues. — Collenchymatic tissue
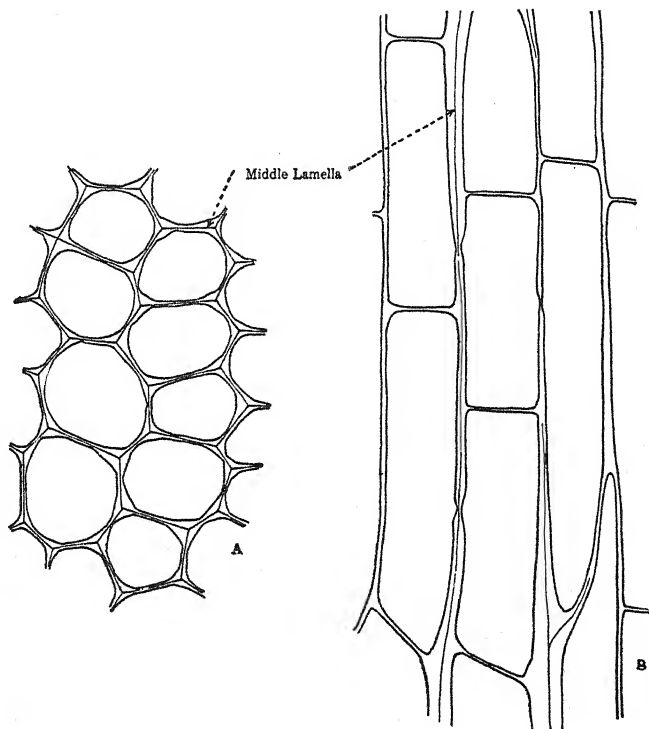
PLATE 48.—Collenchyma Tissue.

A, cross section. *B*, lengthwise section. The cell contents have been omitted.

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may be found in herbaceous or green stems, fruits, seeds and leaves. This element not only serves purposes of support, but is also an assimilation and storage tissue. In the green stems of annual plants little

time is available for the formation of woody tissues in large amounts, nor are these necessary when one considers the short life of such plants. Collenchymatic tissues are more quickly produced and can be so arranged in the herbaceous stem that they serve the purpose of support quite as well as woody tissue. The walls of collenchyma cells are composed of cellulose, but the original cell wall is reinforced by deposition of additional layers of cellulose, until a thick, strong wall is built up. Collenchyma appears in the form of polygonal cells with their walls especially thickened at the angles (Plates 47, 48). The wall is white and of pearly luster. Striations and pores may be present, and the latter may communicate with corresponding pores of an adjacent cell. There is but little intercellular space between collenchyma cells; therefore the cell cavities stand out clearly in a white background of wall substance. One must not mistake these cell cavities for cells, and careful search is often necessary to establish the boundaries of the cell walls surrounding each cavity. In herbaceous stems, particularly the square stems of the Labiatæ or Mint family, the collenchymatic tissue is placed at the four angles of the stem, with perhaps a secondary group between those at each angle. This arrangement places the collenchyma at the points of greatest stress. Collenchyma is usually associated with the fibrous tissues in the midrib of the leaf.

CHAPTER IX

ABSORPTION TISSUES

THE great majority of plants are dependent for their nutrients upon materials contained in the soil and in the air. The nutritive processes of plants differ from those of animals in that the former manufacture nutrients from compounds of very simple chemical composition, as water, oxygen, carbon dioxide and inorganic salts. Animals are ultimately dependent upon the nutrients built up by plants, because the nutrition processes of animals involve a breaking down of the complex substances manufactured by plants. The plant is provided with special organs for the ready absorption of water from the soil and gases from the air.

ABSORPTION OF WATER

As previously noted, the primary root tissues in the dermatogen zone are root cap, root hairs and epidermis. The root cap cells protect the delicate root tissues from injury through contact with sharp soil particles. The cells of the root cap are rather thick-walled and play no part in the absorption of materials from the soil. The epidermal cells of the root are also thick-walled and are more or less impervious to water. The root hairs are directly concerned in the work of absorbing water, which holds in solu-

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tion the salts occurring in the soil. These hair-like structures are located just above the root cap and form a narrow zone between this and the parts of the root covered with epidermis (Fig. 50, No. 1). They are found only on the smallest branches of the root and, although the absorbing surface of each hair is very small, in the aggregate they present an absorbing surface as large and widespread as that of the leaves. Root hairs are similar to trichomes in derivation and are therefore outgrowths of the epidermal cells. Root hairs differ from trichomes in that they are mere slender finger-like projections of the epidermal surface (Plate 49) and the
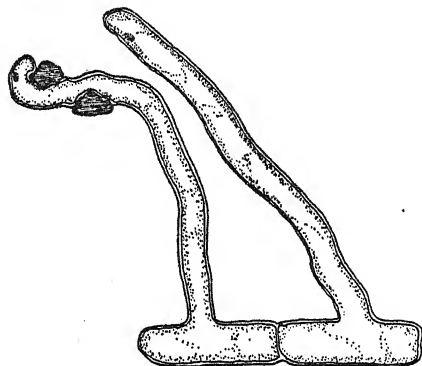
PLATE 49.—The Relation of Root Hairs to Soil Particles. Root hairs in their growth may flatten out over, or partially surround, soil particles.

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projecting portion is not separated from the epidermal cell by a wall. Their walls are extremely thin, and it is possible that the acid reaction, which is apparent when root hairs come in contact with moist litmus paper, is due to an acid secretion. An acid secretion in the vicinity of the root hairs would be very desirable because it would convert many insoluble soil constituents into soluble compounds which are dialyzable. The raw materials consisting of water with dissolved salts dialyze through the thin walls

of the root hairs. They eventually find their way into tubes or vessels through which they are transported to other parts of the plant to be manufactured into assimilable substances. The walls of root hairs are composed of cellulose and many show slight coloration. In the living plant, root hair cells are always in a region of active growth and therefore protoplasmic cell contents are present.

In certain instances the work of absorbing liquids may be performed by plant organs other than root hairs. Plants of parasitic habit and those growing in desert regions show particular adaptability as regards means of absorbing water. *Parasitic plants,* or those which gain their nutrients by burrowing into the tissues of other plants, are usually lacking in root hairs. Absorption of water in this type of plants may take place through the modified epidermis of the aerial roots which are usually present. For their supplies of water, desert plants must depend largely upon dew and very short periods of rain, as the soil in which they live is almost devoid of moisture. Plants living under these conditions have little need for root hairs and these structures are usually lacking. The leaf and stem epidermis of desert plants is so modified that absorption of water can take place through these parts. As the roots of aquatic plants are surrounded by water there is little necessity for an extensive root hair development in these types. Root hairs are very delicate structures and they are seldom found intact in powdered drugs or foods. Where plants are removed from the earth by force, the rootlets bearing root hairs are usually detached and therefore are not apparent.

ABSORPTION OF GASES

Absorption of gases takes place through small openings termed stomata located in the leaf epidermis, and through irregular fissures or lenticels occurring in the periderm of mature plants. The gases entering the plant through stomata or lenticels are distributed to the individual cells by passage through intercellular spaces or by gradual diffusion through thin-walled cells.

Stomata.—Stomata occur in the covering membranes of leaves, herbaceous stems and sepals of the flower (Plate 50). They are occasionally found in the epidermal membranes covering the petals and ovary of the flower. The lower surface of the leaf shows the greatest number of stomata and the upper leaf surface may be entirely devoid of them. The stoma proper is an oval opening perforating the epidermis, and is surrounded by two semicircular cells termed *guard cells*. The guard cells are in turn surrounded by a number of epidermal cells called *surrounding*, *bordering* or *neighboring* cells. In literature on vegetable histology, the term stoma includes the opening in the epidermis together with the two guard cells. The number and arrangement of the surrounding cells is fairly constant for a given species, and this fact may often be used for purposes of identification. The guard cells are modified epidermal cells and usually contain a small number of chloroplasts as a protoplasmic cell content. The stoma may be above, below or on the same level as the epidermal cells. The size of the breathing pore is regulated by contraction and expansion of the guard cells and varies under
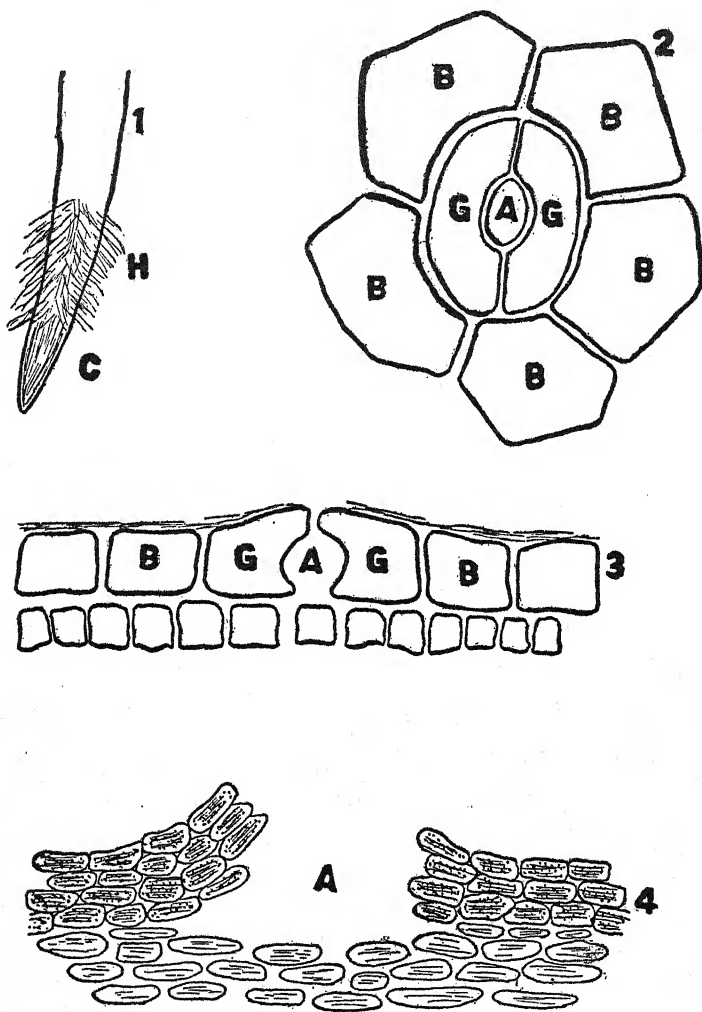
PLATE 50.—Tissues for Absorption.

1. Root hairs (*H*) on rootlet of germinating Fenugrek seed. *C*=root cap.
2. Stomata, surface view. *A*=breathing pore. *G*=guard cells. *B*=bordering, neighboring or surrounding cells. 3. Stomata, sectional view. *A*=breathing pore. *G*=guard cells. *B*=bordering cells. *4.* Lenticel (*A*).

different conditions of light, temperature and humidity of the atmosphere. In general, stomata open when leaves are exposed to light and increased temperatures but they are more sensitive to changes in humidity and close rapidly upon contact with dry air. The latter action is of especial importance in the process of transpiration.

Respiration.—A supply of air is just as necessary for the continuance of life in plants as it is in animals. The air entering the stomata or lenticels contains oxygen and carbon dioxide. The oxygen is essential to cell life and activity, while the carbon dioxide is used by the plant in the manufacture of nutrients. The respiratory processes include the absorption of oxygen through the stomata or lenticels and the distribution of this element to the cells through intercellular spaces or by gradual diffusion through thin-walled cells. Carbon dioxide is one of the waste products resulting from the activity of the cell and is excreted through the stomata. In the consideration of assimilation processes (Chapter XI), it will be noted that carbon dioxide is consumed and oxygen produced, the parts played by the two gases being exactly the reverse of those observed in plant respiration.

Transpiration.—Land plants are constantly excreting or evaporating water through their leaves and other organs provided with stomata, and this process or function is transpiration. The older ideas regarding transpiration were based upon the fact that the mineral salts absorbed by the root hairs are in extremely dilute solution and that, in order to secure appreciable amounts of these inorganic substances, the plant must absorb an enormous amount of water. While water

is a necessary constituent of cell protoplasm the quantity absorbed is far in excess of the needs of the plant and this excess is eliminated through the stomata. Later data, based upon experimental evidence, appear to disprove this theory. It has been shown that great increases in the transpiration rate are not necessarily followed by an increase in the amount of inorganics in the plant. Also researches upon the power of cell walls to absorb different materials at different rates independent of the amounts present would indicate that the absorbing function of the root hairs is not merely a passive or mechanical process. More in line with this evidence is the theory that transpiration is a means of maintaining a circulation of liquids in the plant. The removal of water from the cells of the leaf by transpiration through the stomata results in a concentration of the liquid contents of these cells. According to physical laws, dilute solutions tend to mix with those of greater concentration and so restore an equilibrium. In this manner water containing inorganic salts in the ducts would be drawn toward the leaf cells and these would be assured of a continuous supply of water and other materials for photosynthesis. Incidentally the evaporation or transpiration of water from the leaf surface would tend to prevent excessive heating of these delicate plant organs through exposure to the amount of light necessary to secure maximum photosynthetic activity.

Regulation of transpiration is secured through changes in the size of the breathing pore, and this is in turn dependent upon alterations in the form of the guard cells, possibly due to their water content. When large amounts of water are present in the guard cells

and surrounding tissues, the stoma is wide open so
that water vapor may readily pass from the inter-
cellular spaces to the atmosphere. When the amount
of water in the guard cells is small, they flatten out and
close the stoma, thus preventing evaporation from the
inner tissues.

Water is also eliminated from many plants by spe-
cialized water-secreting organs or *hydathodes*, and by
water pores or water stomata. The removal of water
from the plant body by the hydathodes is an active
secretory process and will be considered under that
heading in Chapter XI. In a few genera, elimina-
tion of water is a passive or filtration process and is
accomplished by openings termed *water pores*, which
are located around the margin of the leaf, and which
communicate directly with the tubular structures
found in the veins of these parts. Water pores are
similar to stomata in structure, but the pore or opening
is not regulated by variations in the size of the sur-
rounding cells and is always open.

Lenticels.—Lenticels are respiratory openings in
the periderm or corky covering tissues of mature
woody plants. These openings expose the tissues
immediately beneath the cork and air thus gains access
to these cells. On surface view the lenticel appears
as an elliptical or oval scar raised above the surround-
ing tissues. Microscopic examination of sections
through a lenticel shows a break or gap in the cork
cells through which the inner bark tissues are exposed
(Plate 50). The inner bark cells in the vicinity of
a lenticel are loosely connected and large intercellular
spaces are apparent. Lenticels are not mere chance
fissures in the bark, but are formed from the meriste-

matic tissue (phellogen) which produces cork. At points of formation the phellogen produces a group of compact cells, which are gradually forced outward until they rupture the existing cork layer, thus giving rise to a lenticel.

CHAPTER X

CONDUCTING TISSUES

THE crude materials in solution absorbed by the root hairs must be transported to the leaves for manufacture into nutrients. The nutrients produced by the leaves must subsequently be distributed to all parts of the plant. In view of these facts the necessity of a system for the conduction or transportation of crude and manufactured materials is clearly apparent. The absorbing tissues are located at the two extremes of the plant, the root hairs at the lower, the leaves at the upper; and communication between these is effected by a system of tubular structures. The conducting tissues are found in all parts of the plant, with the exception of the outer bark, the epidermal membranes and the seed. The outer layers of the bark consist of dead cells; and those cells near the phellogen are nourished by transfusion of nutrients from cell to cell. The epidermal and seed tissues are nourished in the same manner as the cells of the inner bark. The structures concerned in the transportation of material in the plant include ducts, sieve tubes, medullary rays, latex tubes and perforated parenchyma.

Ducts.—Ducts, tracheæ or vessels are continuous tubes extending for considerable distances within the plant body. They are formed by fusion of a vertical

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row of adjacent cells and subsequent absorption of the
end walls of these cells. The walls of ducts are par-
tially lignified and therefore consist of lignocellulose.
As the duct is a structure formed from many cells,
each of which has lost its identity, protoplasmic con-
tents are absent and the tissue is practically lifeless.
Those ducts located in the older portion of the root
may cease to function as conducting elements, owing to
occlusion of the duct lumen through deposition of
resins and other non-dialysable cell contents. Loss
of conducting function may also be brought about
through protrusion of surrounding parenchymatic
cells into the duct cavity. These protrusions of paren-
chyma cells into ducts are termed *tyloses*. Differences
in the arrangement of the lignified substance around the
vessel offer a convenient basis for classification. Lignifi-
cation takes place in a systematic manner, each species
showing peculiarities which may often be used as a
factor in the identification of a given plant. Different
organs of a plant will show different types of vessels,
although the type is definite for each organ. The
types of ducts include pitted, reticulate, scalariform,
annular and spiral forms. *Pitted ducts* are charac-
terized by the presence of numerous pores or thin
places in the lignocellulose wall of the vessel. The
layer of lignin lines all portions of the vessel excepting
the pores, at which points a thin cellulose membrane
permits dialysis of materials. In *reticulate ducts* the
lignified tissue is in the form of a network upon the
inner face of the vessel wall, and the portions not cov-
ered by lignin are irregular in outline. The non-
lignified portions of the walls of *scalariform vessels*
are in the form of long, narrow slits showing fair uni-
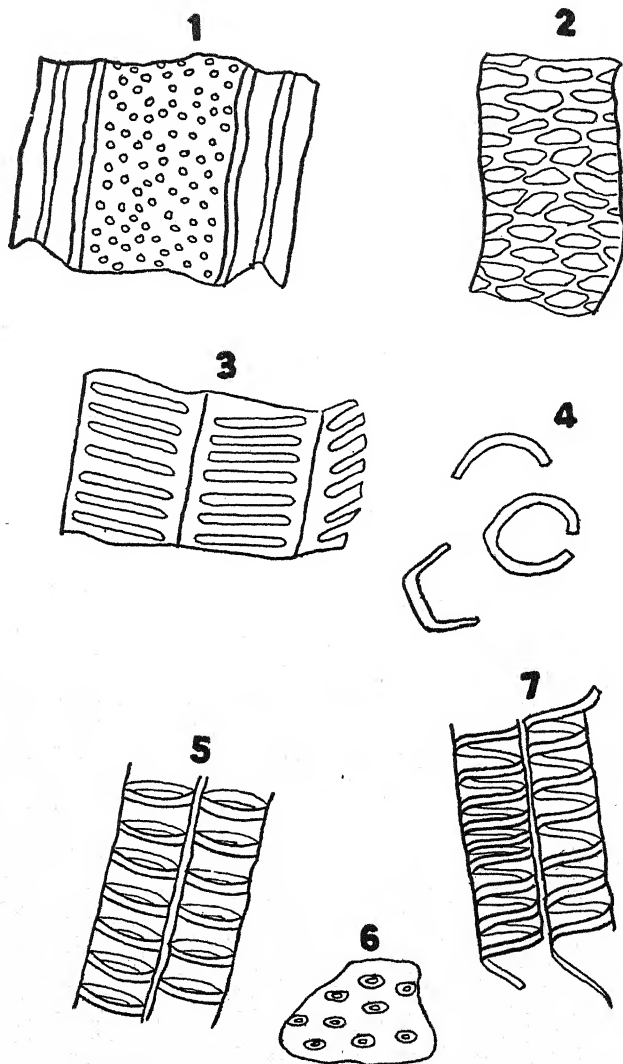
PLATE 51.—Conducting Tissue. Ducts or Vessels.

1. Quassia wood. (Pitted type.) 2. Taraxacum root. (Reticulate type.) 3. Sarsaparilla root. (Scalariform type.) 4. Stramonium leaf. (Fragments of spiral vessels.) 5. Pumpkin stem. (Annular type.) 6. Belladonna root. (Pitted, with bordered pores.) 7. Pumpkin stem. (Spiral type.)

formity both as regards size and arrangement. Scalariform vessels are often angled, and in this respect differ from all other types. Annular vessels are rather thin-walled tubes possessing rings or hoops of lignified tissue within the lumen of the tube. In the *spiral vessels* the lignified tissue is arranged in the form of a continuous spiral band or collection of bands extending throughout the length of the vessel. Lignification in the spiral and annular types is such as to afford flexibility and the maximum of support with the minimum of material, and these forms are usually present in leaves or other parts of the plant which do not survive more than a season.

Tracheids are conducting cells in which the walls between adjacent cells have not been obliterated. Communication between the various cells forming a tracheid is effected by means of pores or thin places in the vessel walls. Tracheids are classified according to the distribution of the lignin, and the terms used in describing ducts may also be applied to tracheids. Frequently the pitted tracheids, and less frequently the pitted ducts, show a line or border around the pore or thin place in the wall. Such structures are known as *bordered pits* or *pores*. The surrounding line is due to local thickening caused by extra deposition of lignin at this point. In other types of bordered pores, a slight thickening also occurs in the center of the cellulose membrane of the pore, which by slight movement against the border may act as a valve. Care should be taken not to confuse tracheids with tracheæ, as the latter structures are ducts or continuous tubular elements.

In general the ducts conduct materials toward the

leaves. The materials contained within the duct are simple substances in aqueous solution and are to be transformed by the leaves into nutrients. Various types of ducts are illustrated in Plate 51.

Sieve Tubes.—Sieve tubes differ from ducts in that they consist of single cells, each of which is a unit in the transportation of materials. The sieve tubes are formed by perforations in the end walls of each cell of a vertical row. These perforated end walls between adjacent cells may be more or less consolidated so that the pores of each wall coincide with those in the walls of the cells above and below. The perforated and partially consolidated end walls are termed *sieve plates*. In transverse sections of cells containing sieve plates the latter appear as yellowish perforated structures entirely filling the cell (Plate 52, No. 2). Upon longitudinal section the true nature of sieve plates is apparent and they are found to be yellowish perforated partitions between cells of a vertical row (Plate 52, No. 1). The older sieve plates are likely to show extremely thick perforated end walls, due to deposition of *callose*, and may contain triangular patches of solidified cell contents. The walls of sieve tubes are composed of cellulose showing no traces of lignification. In general, the sieve tubes conduct materials away from the leaves, the substances transported including nitrogenized nutrients and carbohydrates in solution.

Medullary Rays.—The ducts and sieve tubes provide means for the transportation of materials up and down the stem, and in younger plants communicate directly or indirectly with all the cells. As the stem increases in thickness it becomes necessary to
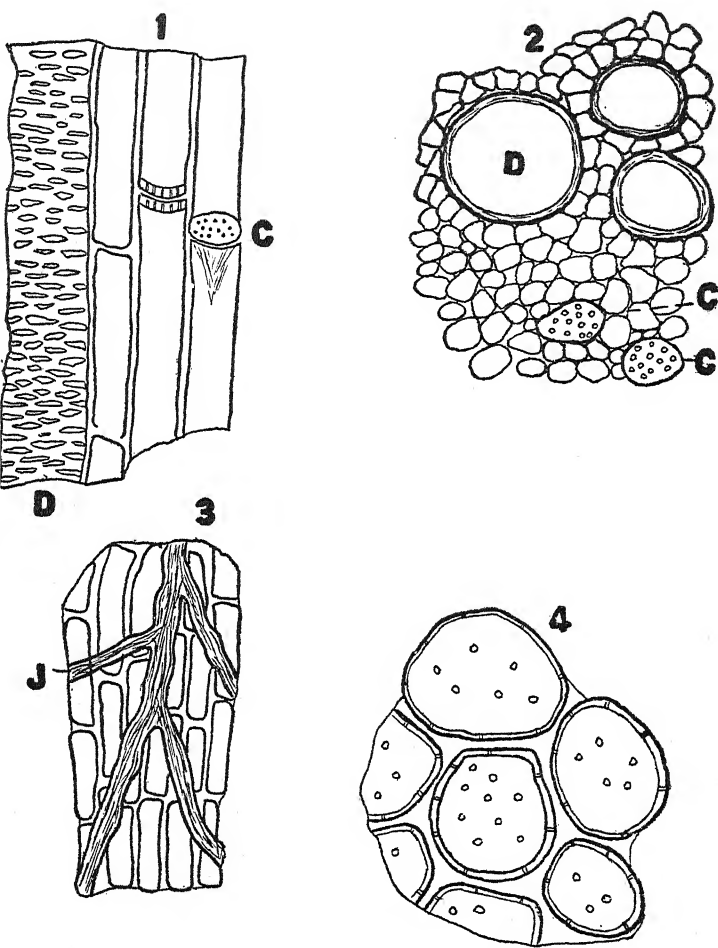
PLATE 52.—Conducting Tissue.

1. Sieve tubes. (Longitudinal view. *D* = duct. *C* = sieve-plate.) 2. Sieve tubes. (Transverse view. *D* = duct. *C* = sieve-plate.) 3. Latex tubes (*J*), Taraxacum root, longitudinal view. *4*. Porous parenchyma, Peppermint stem pith.

provide conducting tissues to transport nutrients to the cells located at some distance from the narrow zone in which the ducts and sieve tubes are placed. This work of lateral conduction is performed by medullary ray cells which extend from the cambium into the bark on one side and into the woody tissues on the other. The first medullary rays are strips of the original parenchyma between the vascular bundles formed in the plerom region, and they extend from the center of the stem to the inner layers of bark without interruption except in the cambium zone. These primary rays are soon supplemented by secondary rays resulting from the formation of new vascular bundles in the widest primary rays. The walls of medullary ray cells are usually thin and are composed of cellulose. In many instances the cellulose walls show numerous pores, thus increasing the efficiency of the ray cells as distributing elements. In the non-porous type of ray cells materials are transported from one cell to another by dialysis through the thin walls. Medullary rays are arranged in groups or bundles, each of which consist of a number of individual ray cells. Upon different aspects these ray groups present striking differences in appearance; and one can readily determine the direction in which a specimen has been sectioned by differences in the appearance of ray cells. Seen in tangential sections, the bundles appear to be elliptical and composed of nearly circular cells (Plate 71*B*). Upon radial view the bundles appear as wide strips made up of large rectangular cells (Plate 71*A*). Upon transverse section the bundles appear as narrow strips composed of one to five rows of rather long rectangular cells

(Plate 70).   In powdered materials the rays are usually seen on radial view.   The contents of medullary ray cells include water,. starch, crystals, resins and tannins.

Latex Tubes.—In certain families of plants conducting tissues known as latex tubes are present. These structures contain and transport a milky juice and derive their name from this fact.   Latex tubes are uninterrupted tubular structures which extend through the various tissues of certain plants.   They may attain great length, are considerably branched and are non-porous (Plate 52, No. 3).   The exact function of this milky juice or latex is not clear, but the close association of the latex tubes with sieve cells and the fact that latex may contain nutritive substances supports a view that these structures are subsidiary conducting elements.   As the juice is usually acrid and purgative, it may be a means of protection against animal attacks.   The latex coagulates upon exposure to air and effectually seals wounds in plant tissues.   Digestive enzymes are contained in the latex of certain plants, notably *Carica papaya,* which contains the proteolytic ferment, papain.   The latex tubes are best viewed in longitudinal sections and appear as irregularly branching structures extending through the parenchymatic tissues.   The contents of latex tubes may become brown in the preparation of specimens and this coloration aids in tracing the course of the vessel.   The walls are very thin and are composed of cellulose.

Porous Parenchyma.—The parenchymatic tissues of the plant may aid in the distribution of nutrients by reason of the fact that nutrients in solution readily dialyze through their thin cellulose walls.   Parenchyma

cell walls, especially those in the pith or central region of the plant are usually perforated (Plate 52, No. 4); and the transfer of liquids through these cells is fairly rapid.

FIBRO-VASCULAR TISSUES

As the tissues concerned in the transportation of nutrients are usually delicate structures, they are supported by fibrous elements. Each group of vessels, with the adjacent mechanical or supporting tissue, is termed a fibro-vascular bundle. As previously noted in Chapter VI, the fibro-vascular bundles may be complete or incomplete. The complete bundles consist of xylem and phloem, and cambium elements may or may not be present, whereas the incomplete bundles consist of either xylem or phloem tissues, cambium being absent. The xylem bundles consist of a vascular element, ducts or vessels, supported by a fibrous element, xylem or wood fibers. The phloem bundles likewise consist of a vascular element, sieve tubes, supported by a fibrous element, bast fibers.

The tissues composing a fibro-vascular bundle may be arranged in any one of several different ways. Because of these differences in arrangement, five types of bundles are recognized. *Radial fibro-vascular bundles* are always incomplete, consisting of xylem *or* phloem. They are further characterized by the arrangement of xylem or phloem elements in a circle within the endodermis, and the alternation of each xylem bundle with a phloem bundle. Radial bundles are found in all young roots and may even be present in mature monocotyledonous roots. They are an
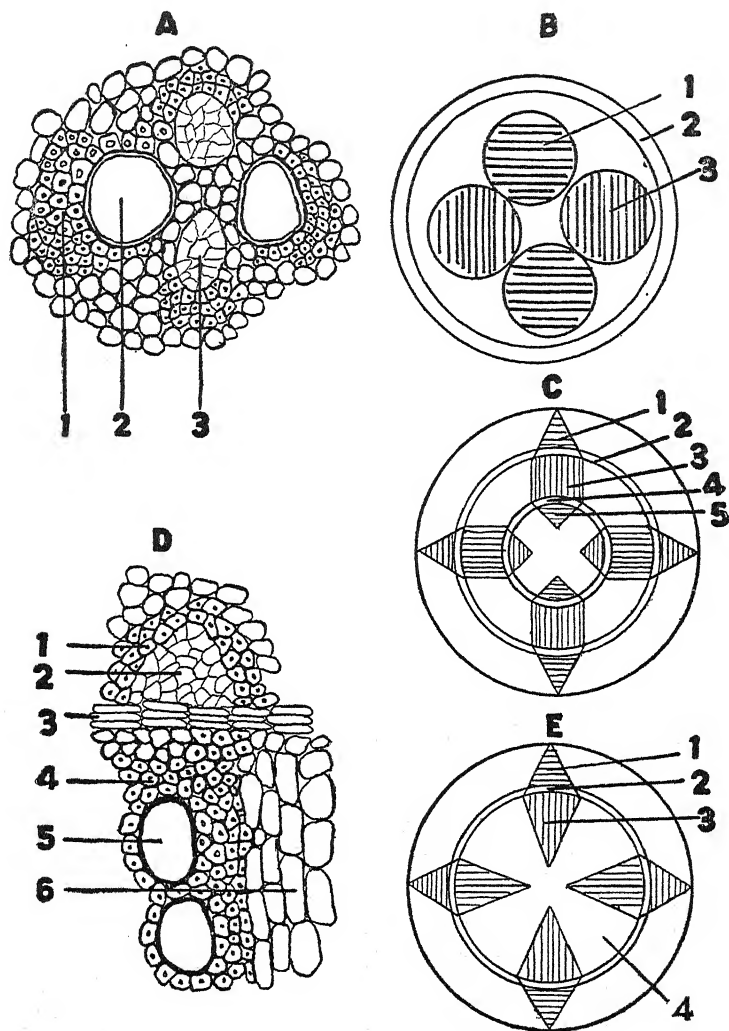
PLATE 53.—Fibrovascular Bundles.

A. Collateral type, Bamboo stem. 1. Fibrous tissue. 2. Ducts. 3. Sieve.
B. Collateral Bundle, arrangement of fibrovascular elements. 1. Xylem.
2. Endodermis. 3. Phloem.
C. Bicollateral Bundle, arrangement of fibrovascular elements. 1. Phloem.
2. Cambium. 3. Xylem. 4. Cambium. 5. Phloem.
D. Open collateral type, Aconite tuber. 1. Bast fibers. 2. Sieve cells. 3. Cambium. 4. Wood fibers. 5. Ducts. 6. Medullary ray.
E. Open Collateral Bundle, arrangement of fibrovascular elements. 1. Phloem.
2. Cambium. 3. Xylem. 4. Medullary ray.

indication of primary structure in those plant parts in which they occur. *Concentric fibro-vascular bundles* are complete bundles consisting of xylem *and* phloem. They are characterized by the arrangement of xylem and phloem elements in such a way that one of these surround the other. Most frequently the xylem surrounds the phloem, and the bundles are scattered irregularly in a central pith region within the endodermis. This type of bundle occurs only in monocotyledonous roots and stems. *Collateral fibro-vascular bundles* are of the complete type and show xylem *and* phloem elements, together with cambium arcs. There are three types of collateral bundles, closed collateral, open collateral, and bi-collateral. *Closed collateral bundles* consist of one or more xylem portions and phloem portions separated from each other by a short strip of cambium or by parenchymatic elements, the whole being surrounded by a sheath or layer of fibrous tissue. The bundles may be scattered irregularly in the pith and, as a rule are only present in monocotyledonous rhizomes or stems. *Open collateral bundles* consist of xylem elements within a cambium zone and phloem elements on the outer side of the cambium. When occurring in roots, this type of bundle may represent secondary development of the primary radial bundles. In stems, this type of bundle may be completely developed even in the primary stages of growth. The open collateral bundle is the type most frequently found in mature dicotyledonous roots and stems. *Bi-collateral fibro-vascular* bundles consist of a xylem element with associated cambium and two phloem elements, one on each surface of the xylem. This
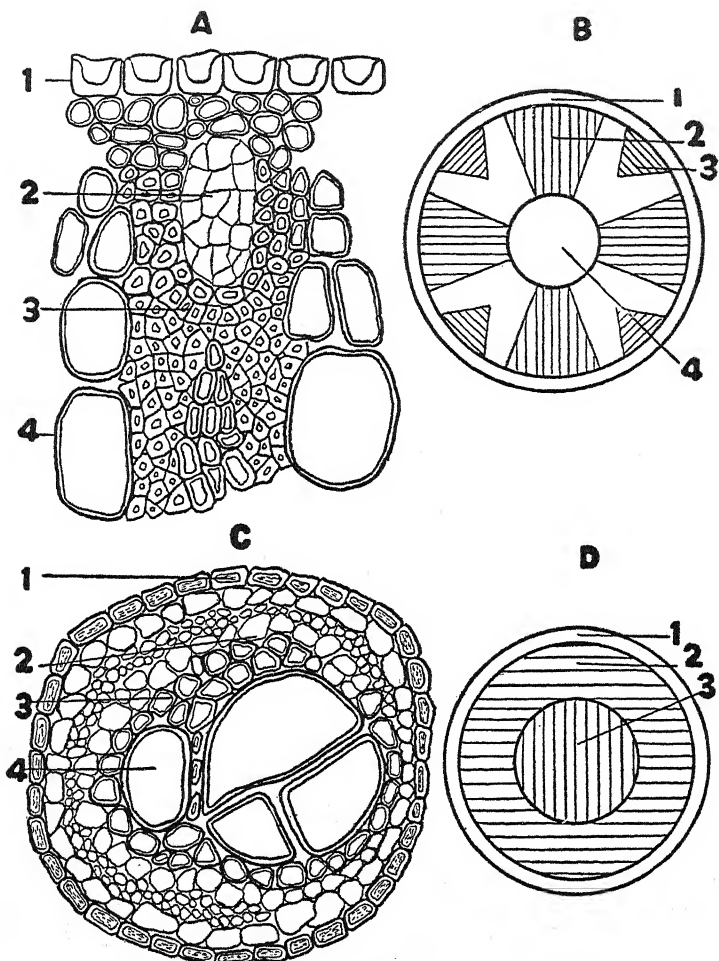
PLATE 54.—Fibrovascular Bundles.

A. Radial type, Sarsaparilla root. 1. Endodermis. 2. Sieve surrounded by bast fibers. 3. Wood fibers surrounding sieve and ducts. 4. Ducts.

B. Radial Bundle, arrangement of fibrovascular elements. 1. Endodermis. 2. Xylem. 3. Phloem. 4. Pith.

C. Concentric type, Fern rhizome. 1. Endodermal sheath. 2. Sieve surrounded by small parenchyma. 3. Fibrous tissues. 4. Ducts.

D. Concentric Bundle, arrangement of fibrovascular elements. 1. Endodermal sheath. 2. Phloem. 3. Xylem.

type of bundle is present in but few mature dicoty-
ledonous roots and stems.

The various types of fibro-vascular bundles are
illustrated in Plates 53 and 54. The arrangement
of the different elements in each type of bundle is
represented in the diagrams accompanying these
illustrations.

CHAPTER XI

TISSUES FOR SYNTHESIS, ASSIMILATION AND STORAGE

THE plant possesses the power of building up the very simple chemical compounds absorbed through the root hairs and stomata into nutrients of complex composition. In other words, the plant must construct its nutrients before assimilating them. This procedure is quite different from the nutrition process in animals, as the food materials of the latter consist of complex nutrients which undergo analytic changes in the organism.

SYNTHESIS OF NUTRIENTS

Synthetic processes occur in many organs of the plant; but the most important are those occurring in the leaves and green stems. Starch is the most important plant nutrient; and, as chloroplasts are responsible for its formation, the synthesis of this substance occurs only in the green parts of the plant. As the chemical changes occurring in the formation of starch are initiated by, or are dependent upon, the action of light rays, the process is termed a *photo-synthesis*. The crude materials entering into the formation of starch are water and carbon dioxide. These simple substances are built up into the complex starch molecule by the chloroplasts. There is much

controversy over the exact chemical reactions involved, and the two sets of reactions given represent possibilities rather than actualities.

1. $$CO_2 + H_2O = HCOOH + H_2O_2;$$
$$\text{formic acid}$$

$$x(HCOOH) = (CH_2O)_x;$$

$$6(CH_2O) = C_6H_{12}O_6;$$
$$\text{glucose}$$

$$(C_6H_{12}O_6)_x = (C_6H_{10}O_5)_x + (H_2O)_x.$$
$$\text{starch}$$

2. $$6CO_2 + 5H_2O = C_6H_{10}O_5 + 6O_2.$$
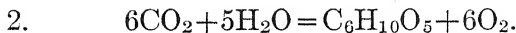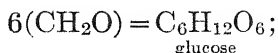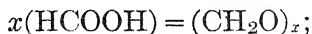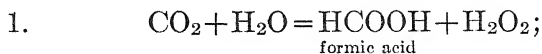
In the first set of reactions formic acid is formed from carbon dioxide and water. An unknown number of molecules of this acid is transformed into a substance having the formula of a typical carbohydrate (CH_2O). This carbohydrate substance, by rearrangement of the elements composing it, is transformed into glucose. The glucose is converted into starch by the elimination of a molecule of water. The second reaction assumes a direct formation of starch from the carbon dioxide and water with the liberation of oxygen. The weight of opinion inclines toward the building up of a series of intermediate compounds as indicated in the first reaction. Chloroplasts and light are essentials in both reactions.

Although the exact nature of the chemical changes occurring in the construction of starch substance are in doubt, the manner in which the starch granule is built up by the chloroplast is fairly well understood. The chloroplasts are protoplasmic cell con-

tents and appear as small oval disks of about constant size. Each chloroplast owes its green color to the substance *chlorophyll*. The chloroplasts are found in the inner tissues of the leaf, being especially numerous in the cells immediately beneath the epidermal membrane. The starting point in the formation of a starch grain by the chloroplast is the appearance of a small projection or bud upon the latter. This projection increases in size as the starch substance is deposited around it, and may become much larger than the chloroplast. The newly formed starch grain eventually becomes detached from the chloroplast. In daylight the formation of this *assimilation starch* by the chloroplasts proceeds with great rapidity, and the starch must be removed from the place of synthesis to make room for further production. Starch is a colloidal or non-dialyzable substance; therefore, before it can pass through the cell membranes of the leaf tissues, it must be converted into a soluble or dialyzable form. Enzymes present in the leaf cells dissolve the newly formed starch, converting it into *soluble starch* which can dialyze through the cell walls. This soluble starch is transported by the conducting tissues to different parts of the plant where it is used as a nutrient or stored against future needs.

Frequently the greater part of the soluble starch is stored in well-protected parts of the plant. The soluble starch or glucose transported from the leaves may be stored in the form of reserve starch, inulin, sucrose, glucosides or cellulose. The leucoplasts take part in the formation of storage or *reserve starch* from the soluble starch, and the process is somewhat similar to that occurring in the formation of assimilation

starch. A bud appears upon the surface of the leuco-plast, and the soluble starch is deposited in successive layers around this bud. The starch grain increases in size, becoming larger than the leucoplast and is finally detached from the latter. Certain mark-ings upon the starch grain have direct connection with the processes of formation. The first part of the starch grain to be produced is termed the *hilum* and is often of characteristic form. The different layers deposited around the hilum are often apparent through the presence of *striations* or markings upon the face of the grain. One or several starch grains may be developed from one leucoplast, thus giving rise to *simple* and *compound* forms. Several types of starch grains are illustrated in Plate 57.

Inulin, instead of reserve starch, is formed from the soluble starch or carbohydrate in a few families of plants. Inulin is a reserve material and is probably merely deposited in the cells, the leucoplasts taking no part in the process.

Sugars.—The formation of sucrose or cane sugar from the soluble starch or glucose takes place in the stems of sugar cane and the roots of the sugar beet. Sucrose is a reserve material and, because of its solu-bility, is always in solution form, only crystallizing upon desiccation.

Aleurone.—Little information is available on the formation of the albuminous or nitrogenized nutrients of plants. They are possibly formed by interaction between the soluble carbohydrates produced in the leaves and the soluble inorganic salts absorbed through the root hairs. This synthesis may be carried out in the leaf cells and in the meristematic regions of

the plant. *Aleurone* is nitrogenized reserve material and is usually stored in those seeds which contain large amounts of oil but are lacking in starch. In the synthesis of nitrogenized nutrients, oxalic acid may be formed, and this poisonous substance is possibly disposed of by interaction with the calcium salts present, resulting in the formation of calcium oxalate, which because of its insolubility is practically harmless. Calcium oxalate occurs in various crystalline forms and is of service in the identification of certain vegetable materials.

Hydrocarbons.—This group includes the vegetable fats and oils, all of which are esters or organic salts of the fatty acids, chiefly palmitic, stearic and oleic. There is a possibility that reserve substances of this class may be produced by a complex series of syntheses from the soluble carbohydrate formed in leaves. Fats are frequently associated with aleurone and are present in the cell as minute droplets or in solid form.

Glucosides.—These are substances which, upon digestion with dilute mineral acids, readily decompose, yielding glucose as one of the products of the reaction. Many glucosides are extremely poisonous. Aside from the fact that they may protect the plant against animal attacks, little is known of their exact function in the plant economy.

Reserve Cellulose.—The soluble carbohydrates may be transformed by substances within the cell into reserve cellulose. The reserve cellulose is deposited upon the original thin cellulose wall of various cells, resulting in the formation of collenchymatic tissue.

SECRETING CELLS AND CAVITIES

Secreting cells are concerned in the synthesis of resins, gums and volatile oils. While these substances are formed from the products of assimilation they are not as intimately concerned in the nutrition of the plant as are the substances noted in the preceding section. The plant tissues performing this function of secretion include glandular hairs, the walls of secretion cavities, individual secretion cells and oil ducts.

Glandular Hairs.—The histological forms which glandular hairs may assume have been described in the section on Plant Hairs (Chapter VII). Glandular hairs are outgrowths of the epidermal tissues and possess glands consisting of one or more cells, which have the power of secreting or producing volatile oils or resins. In some instances, the gland cavities are formed by a separation of a thin membrane from the upper surface of a number of modified epidermal cells; in others no special cavity is apparent, and the secretions are evidently stored within the gland cells. Glandular hairs may occur on leaves, herbaceous stems and the petals of the flower. The secretions include volatile oils, resins or oleoresins.

Secretory Cavities.—Secretory cavities may occur in the internal tissues of leaves, barks, roots, woods, fruits and seeds. According to the manner of their formation they are subdivided into schizogenous and lysigenous types. *Schizogenous cavities* are formed through enlargement of the intercellular spaces in the region adjacent to the secreting cells (Plate 55, No. 2). The secretory products dialyze into the
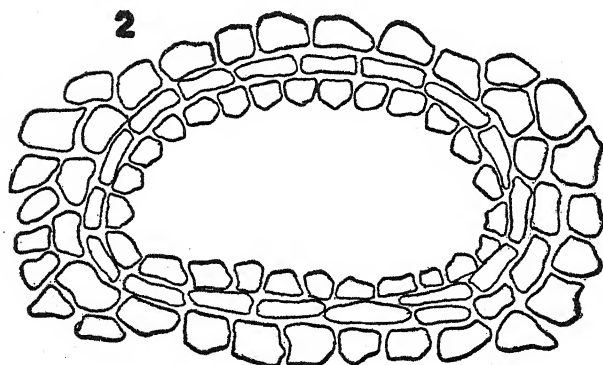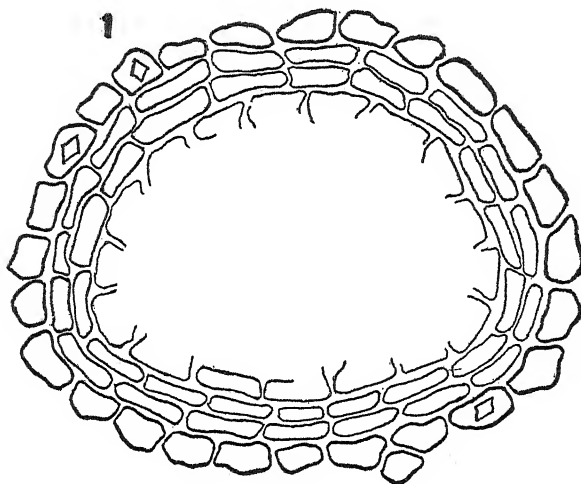
PLATE 55.—Secretion Cells.
1. Lysigenous cavity, Orange peel.   2. Schizogenous cavity, White Pine bark.

intercellular space and cause distention of the latter. The mature schizogenous cavity appears to be completely surrounded by a layer of secreting cells. *Lysig-*
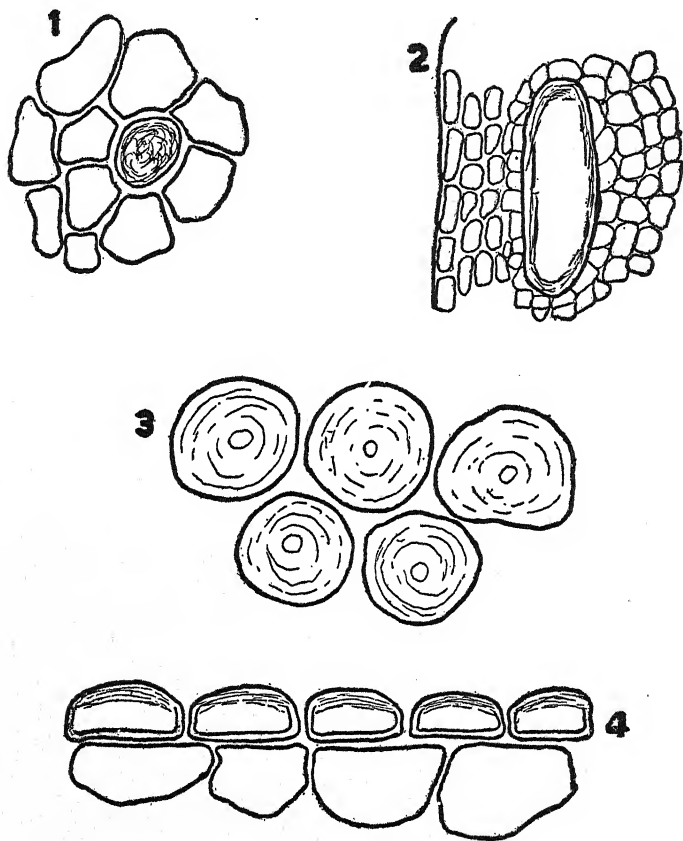
PLATE 56.—Secretion Cells.

1. Unicellular secretion cells, Galangal rhizome. 2. Vittæ, secretion cavities, Fennel fruit. 3. Mucilage cells. Mustard seed. (Surface view.) 4. Mucilage cells, Mustard seed. (Sectional view.)

enous cavities result from the disintegration of a group of secretory cells. This is brought about through dis-

tention of the secreting cells by accumulated secretions. The cells finally burst and discharge their contents into a common cavity (Plate 55, No. 1), Lysigenous cavities usually show traces of the partially disintegrated cells in the form of projecting fragments of wall.

Unicellular Secretion Cells.—Individual cells may function as secreting organs. Unicellular secretion cells differ from secretion cavities and oil ducts in that the secretion remains within the individual cell and is not stored in a special cavity (Plate 56, No. 1). Secretion cells may show but little differences in size and form from the surrounding cells, except as the secretion displaces the protoplasmic contents or accumulates and distends the cell walls. In certain types of unicellular secretion cells, the secreted products are apparently stored within sacs especially formed for the purpose. The materials produced by these cells include oils and oleoresins.

Vittæ.—Oil channels or vittæ are especially characteristic of the Umbelliferous fruits. They are located in the fruit parenchyma or mesocarp and appear as irregular, schizogenous, duct-like structures extending the full length of the fruit (Plate 56, No. 2). The number of vittæ present is a diagnostic character of interest in the differentiation of closely related species. Volatile oils are the most important products secreted by these oil ducts.

WATER SECRETION

In certain plants, elimination of water is accomplished by specialized secretory organs termed *hydathodes*. These structures may or may not be in direct communication with the water-conducting ele-

ments, and both unicellular and multicellular forms occur. The simplest hydathodes are merely modifications of the individual cells of an epidermal membrane. More complex types assume hair-like form, and still other types consist of groups of modified epidermal cells in contact with loose parenchyma in which vascular elements end. Mucilage formation is frequently a subsidiary function of the secreting cells of the hydathode and is a means of attracting water from the surrounding tissues.

STORAGE TISSUES

The period of greatest activity in the manufacture of nutrients is the summer season. Perennial plants must lay by a store of food to carry them over the winter months in which the production of nutrients practically ceases. The tissues concerned in storing nutrients include parenchyma cells, secretion cavities, collenchyma cell walls and the cavities of fibers and stone cells.

Parenchyma.—The parenchyma cells of the cortical and pith regions are the chief storage places for plant nutrients. Mention has already been made of the formation of starch in the parenchyma cells by the leucoplasts. This reserve starch is stored in the parenchyma of well-protected parts of the plant. Inulin, while in solution in the living plant, may become deposited through desiccation and is similarly stored in the parenchyma of tubers and roots. Alkaloids and calcium oxalate crystals may also be stored in the parenchyma cells, while tannins are frequently found in bark parenchyma cells.

Secretion Cavities.—The volatile oils or other products of secretory cells are usually stored in cavities of lysigenous or schizogenous types or may be contained in the gland cells.

Collenchyma Cell Walls.—The walls of many cells, especially those of seeds and fruits, are thickened by deposition of cellulose. This cellulose may be classed as a reserve nutrient and may possibly be converted into assimilable form if required. Collenchyma is thus a tissue of synthesis, a supporting tissue and a storage tissue.

Cavities of Stone Cells and Fibers.—Storage of nutrient material within the cavities of stone cells and fibers occurs in comparatively few instances. Materials stored in these thick-walled cells are not as readily available for use as nutrients as are those contained in the thinner-walled parenchyma.

CHAPTER XII

CELL CONTENTS

CELL contents, or substances resulting from the activity of the protoplasm, may be classified according to their chemical constitution and also according to their structure or form. Classifications based upon structure or form are better adapted to histological work than those founded solely upon chemical characters. Microchemical tests for these various cell contents will be found in Chapter IV. The classification which follows is based primarily upon structural characters, and physical differences are used as a secondary point in the grouping.

Cell Contents of Definite Form
　Colloidal Group:
　　Starch
　　Inulin (upon treatment with alcohol)
　　Aleurone
　Crystalline Group:
　　Sugars (upon desiccation)
　　Alkaloids (upon formation of alkaloidal salts)
　　Glucosides
　　Calcium Oxalate
Cell Contents of Indefinite Form
　Colloidal Group:
　　Inulin (upon desiccation)
　　Tannins
　　Gums
　　Resins
　　Fats and Oils
　　Silica Deposits
　　Calcium Carbonate Deposits

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Starch.—The transitory or assimilation forms of starch described in Chapter XI are of little importance in food and drug microscopy; and it is the reserve or storage starch, the grains of which are fairly constant in a given plant, which will be considered in this work. Starch grains may be classified according to the following characters:

> Number of grains in the mass;
>> single, compound and aggregate.
>
> Shape or form of the grains;
>> circular, oval and angular forms.
>
> Position of the hilum; ·
>> centric or excentric.
>
> Shape or form of the hilum;
>> point, line and stellate forms.
>
> Presence or absence of striations;
>> striated and nonstriated forms.
>
> Size of the grains;
>> ranging from 2 to 100 microns.

The leucoplast produces either single starch grains or masses consisting of several grains. In the latter instance the cluster is termed a *compound grain*. The shape or form of a starch grain depends upon the number of grains developed from each single leucoplast. The parts of a compound grain are usually angled because of the presence of surrounding grains which exert pressure on all sides. Grains developed in groups of two or three will be flattened on the sides in contact with other grains and rounded on the free surfaces. *Beaked* forms show a small projection at the hilum end of the grain. *Starch aggregates* usually represent the entire contents of a parenchyma cell and consist of innumerable small angled grains, which adhere even after milling to fine powder.
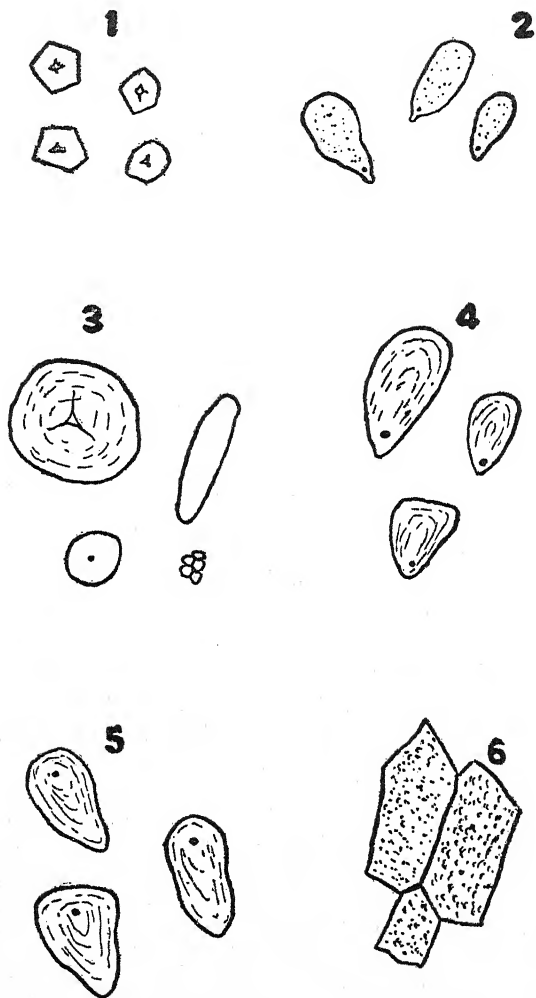
PLATE 57.—Cell Contents.   Starch Grains.

1. Corn starch. (Angled grains, centric stellate hilum.) 2. Ginger starch. (Beaked grains, excentric point hilum.) 3. Rye starch. (Circular grains, centric fissured and point hilum.) 4. Potato starch. (Ovoid grains, excentric point hilum.) 5. Maranta starch. (Ovoid grains, excentric point hilum.) 6. Pepper starch. (Compound grains.)

Starch grains of a given plant range in size within fairly constant limits. In stating the sizes of compound grains, the component parts as well as the whole mass should be considered. Compound grains in powdered materials are always more or less broken, and it may be difficult to secure satisfactory views unless one examines sections.

The position of the hilum is dependent upon the manner in which the grain is formed by the leucoplast and upon the portion of the grain viewed by the observer. In view of the latter fact one cannot readily determine the position of the hilum in materials examined in temporary mounts until the particles have come to rest. The polarizing microscope is very useful in determining the position of the hilum, as this is always located at the intersection of the dark bands that are apparent on viewing starch grains with crossed prisms.

The form of the hilum may be modified by the amount of moisture in the grain. Fissures may arise from a *point hilum* through drying. A *stellate hilum* shows several radiating fissures. A *line hilum* is formed by a straight fissure arising from the hilum. A *V-shaped hilum* results from the formation of two straight fissures from the hilum.

Striations, when apparent, always encircle the hilum, and are best observed in water mounts. A number of typical starch grains are illustrated in Plate 57.

Inulin.—In dried roots and tubers this substance occurs in flat tabular masses, but upon treatment with alcohol the inulin in these plant parts will separate in spheroidal masses closely resembling certain forms

of starch. The tabular forms are usually much broken and very irregular (Plate 58, No. 2). Inulin grains or masses do not possess a hilum nor do they show striations. The spheroidal and starch-like forms may show fissures extending from the center of the mass (Plate 58, No. 1).

Sugars.—The sugars contained in plant cells are usually in solution and are therefore not visible unless the cell liquids become so concentrated that crystallization takes place.

Alkaloids.—The alkaloids are seldom visible in plant cells and their presence is usually demonstrated by the addition of mineral acids which form crystalline salts.

Glucosides.—Glucosidal substances may or may not be visible in plant cells without special treatment. Those glucosidal substances which do occur in visible form show such great variations in character that general descriptions cannot be given. The deposits of the glucoside, hesperidin, in buchu leaves are illustrated in Plate 58, No. 3.

Calcium Oxalate.—This material may occur in almost any part of the plant and, with the exception of starch, is perhaps the most important cell content from the standpoint of the analyst. Calcium oxalate occurs in needles, rosettes and various other crystalline forms. The types of crystals recognized are acicular, rosette or aggregate, prismatic and microcrystals (crypto-crystalline crystals). *Acicular* or *needle-like crystals* may occur singly or in bundles termed *raphides*. *Rosette crystals* are aggregates of small prismatic forms. The *prismatic* forms of calcium oxalate are modifications of the monoclinic and
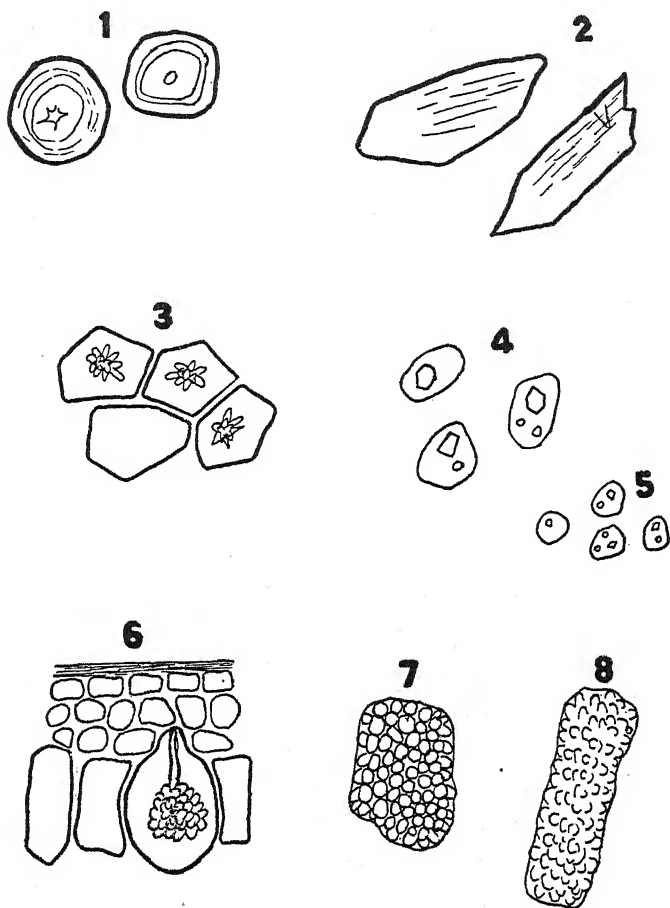
PLATE 58.—Cell Contents.

1. Inulin, spheroidal masses from Dahlia root. 2. Inulin, tabular masses from Echinacea root. 3. Hesperidin, deposits in epidermal cells, Buchu leaf. 4. Aleurone, Ricinus seed. 5. Aleurone, Mustard seed. 6. Cystolith, in subepidermal cells, Ficus leaf. 7. Cystolith, Nettle root. 8. Cystolith, Ruellia root.

tetragonal systems. Perfect crystals are the exception rather than the rule, although those of cubical or monoclinic form may be fairly symmetrical. *Micro-crystals* are the smallest type of plant crystal, and, although they are usually plentiful when present, are often difficult of recognition because of their small size. These crystals occur as triangular prisms and appear as V-, Y- and T-shaped forms, according to the crystal angles exposed to view. *Crystal sand* consists of minute micro-crystals or the broken fragments of larger crystals, and occurs in many powders. In materials containing sand or earth, care must be taken not to confuse crystalline quartz particles with calcium oxalate crystals. These sand crystals are always very irregular in form and usually show scratches or abrasion marks on the surface. Various types of plant crystals are illustrated in Plate 59.

Tannins.—These materials occur as brownish or blackish masses without characteristic form.

Gums.—The gum deposits may fill the entire cell and are usually observed by examining the material in an aqueous mounting medium which causes swelling of the contained gum. The mucilage formed upon the addition of water appears in the form of highly refractive globules, which remain light colored after the diaphragm is closed (Plate 56, Nos. 3 and 4). It is noteworthy that parenchyma cells containing raphides often contain gum, and in fact the mass of crystals may be embedded in the mucilaginous contents.

Resins.—Resin deposits in plant cells differ greatly in color and lack definite form.

Fats and Oils.—These substances frequently appear as highly refractive globules which may be mis-
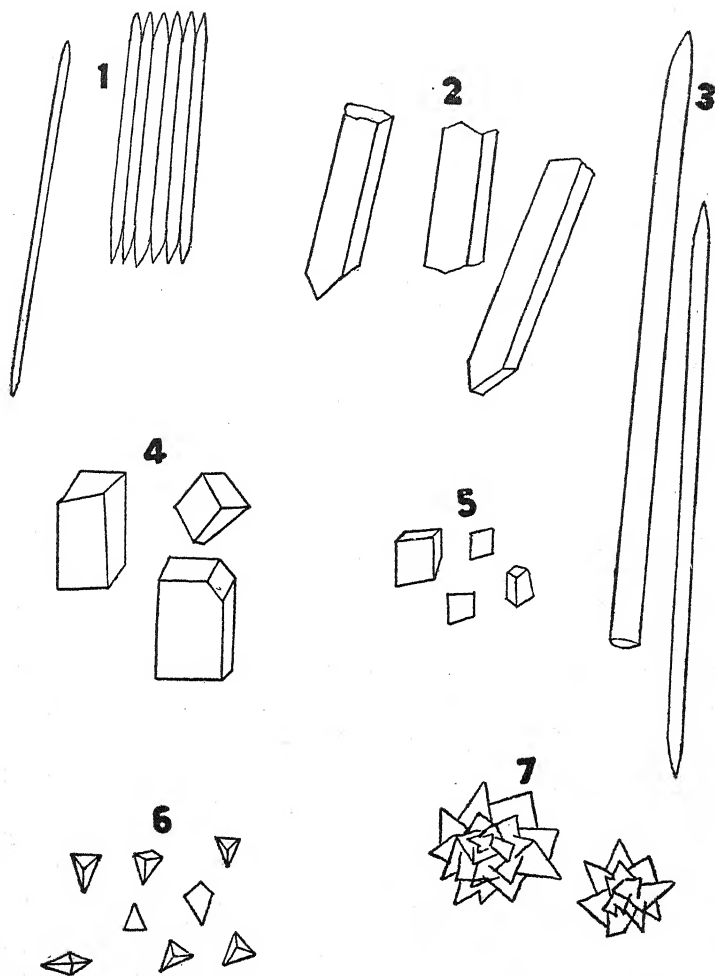
PLATE 59.—Cell Contents.

1. Acicular crystals, single and in raphides, Sarsaparilla root. 2. Prismatic crystals, Orris root. 3. Acicular crystals, Squill. 4. Prismatic crystals, Prunus bark. 5. Cubical crystals, Senna leaf. 6. Cryptocrystalline crystals, Belladonna root. 7. Rosette crystals, Euonymus bark.

taken for air bubbles in well-lighted fields. When the diaphragm is closed, the outer margin of an air bubble increases in thickness, while that of an oil globule does not.

Silica Deposits.—This material occasionally occurs in epidermal and fiber cells. The deposits appear as white, spherical bodies with numerous small spines or projections upon their surface.

Calcium Carbonate.—This substance appears in grayish masses of indefinite form and may possibly be mistaken for compound starch grains. The deposits are termed *cystoliths* and are composed of numerous small globular particles of carbonate (Plate 58, Nos. 6, 7, 8). Aside from the absence of a hilum in the small globules forming the masses, they may be distinguished from starch by their solubility in mineral acids with liberation of carbon dioxide.

Aleurone.—Aleurone granules occur in several different forms and may closely resemble small starch grains. A form frequently seen consists of globular bodies, each containing one or more crystalline structures, or having the latter attached to the outer edge of the grain. The crystalline particles occurring in connection with the aleurone grains may be composed of calcium oxalate, but in many instances they are substances responding to the usual protein tests. Other forms show globular bodies within the grain or attached to its outer edge. The iodine reaction may be used to distinguish between starch and aleurone grains, as the latter become brown upon addition of the reagent. Various types of aleurone grains are illustrated in Plate 58, Nos. 4 and 5.

CHAPTER XIII

ROOT STRUCTURES

In considering the histology of roots it is noted that the roots of many classes of plants undergo the changes noted in Chapter VI, resulting in the development of secondary or permanent tissues. Other types of roots do not undergo this series of changes incidental to the production of secondary structures. Therefore it is convenient to subdivide root structures into those found in secondary roots and those present in primary roots. The roots of most monocotyledons retain their primary structures throughout the life of the plant. Dicotyledonous roots, while exhibiting primary structures during the earlier stages of growth soon undergo modification resulting in the formation of secondary structures. The chief differences between the primary and secondary types of roots are in the nature of the covering tissues, the arrangement of the fibro-vascular bundles, and the presence or absence of a distinct endodermal layer.

PRIMARY ROOT STRUCTURES

Primary root structures are present in the earlier stages of root development of both monocotyledons and dicotyledons; but, in the former class of plants, primary structures are retained throughout the life of the individual. The tissues usually present in roots
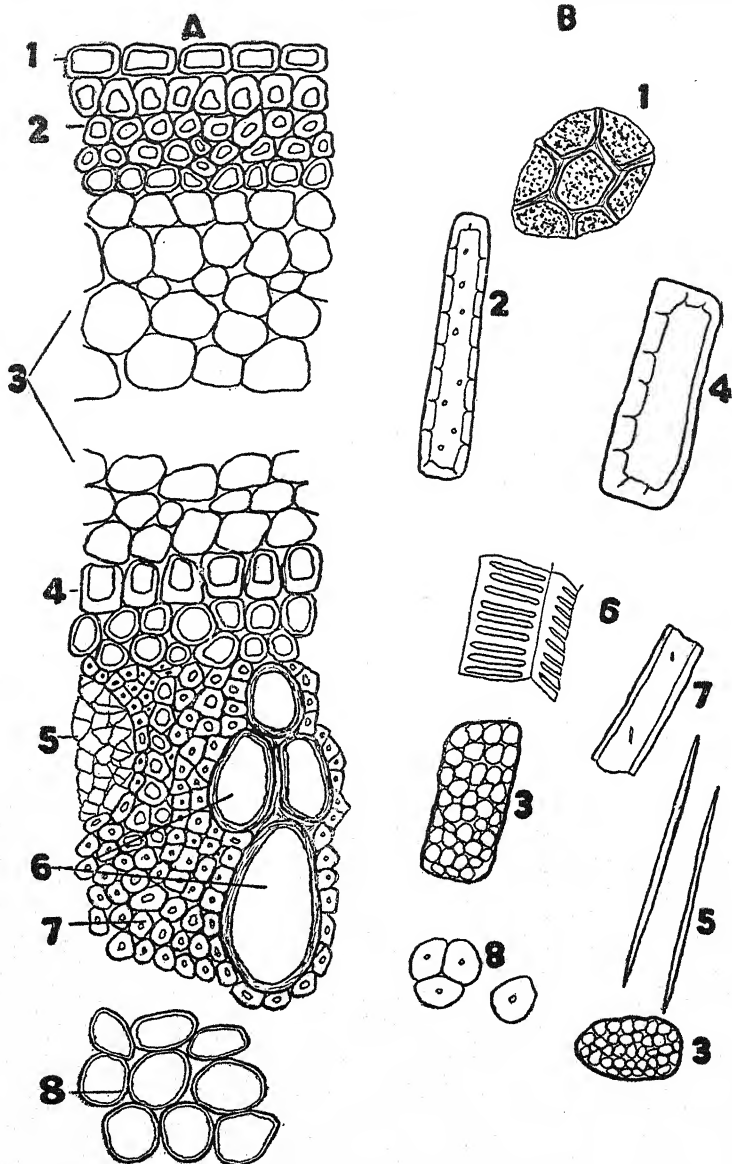
PLATE 60.—Primary Root Structure.

A. Transverse Section, Sarsaparilla root. 1. Epidermis. 2. Hypodermis. 3. Cortical Parenchyma. 4. Endodermis. 5. Sieve tubes. 6. Ducts. 7. Fibers. 8. Pith.

B. Powdered Sarsaparilla root. 1. Epidermis. 2. Hypodermis. 3. Parenchyma, longitudinal and transverse views, cells filled with starch. 4. Endodermis. 5. Acicular crystals. 6. Scalariform vessels or ducts. 7. Fibers. 8. Starch grains, single and compound.

of primary structure and the order in which they occur, beginning at the outermost layer, are as follows:

1. Epidermis,
2. Hypodermis,
3. Cortical parenchyma,
4. Endodermis,
5. Phloem bundles,
6. Xylem bundles,
7. Pith parenchyma.

By reference to Plate 60 it will be seen that each of these tissues possesses certain structural characteristics and fairly definite cell contents whereby it may be distinguished from the others even when the material is finely powdered. In the identification of unknown samples of roots, it is important to determine whether the material is from a monocotyledonous or a dicotyledonous plant. It is extremely unlikely that the younger dicotyledonous roots showing primary structure would be present in such material. Therefore the presence of primary tissues in vegetable substances is a fairly certain indication that the material is from a monocotyledonous plant.

CHARACTERS OF THE PRIMARY ROOT TISSUES

Epidermis.—The epidermal cells of mature primary roots are usually darker colored than the other tissues. Root hairs or peripheral elongations of the epidermal cells are often present. These structures are integral parts of the epidermal cells and must not be confused with trichomes or plant hairs occurring on over-ground portions of the plant. The root hairs are usually slightly colored, like the epidermal cells, and are extremely thin-walled so as to permit ready

absorption of dialyzable materials. The epidermis is seldom more than one layer of cells in thickness, and the cells are not strongly cutinized as are those of the green portions of the plant. As illustrated in Plate 60, epidermal cells in sectional view appear in more or less rectangular or oblong forms. They are rather thin walled, excepting upon exposed surfaces, which may show slight thickening. On surface view, which is the most frequently had when examining powdered materials, epidermal cells show rectangular and polygonal forms.

Hypodermis.—The hypodermal layer occurring in primary roots is immediately within the epidermis; and the individual cells are usually slightly colored. This tissue may be one or more layers of cells in thickness, but the number of cell layers seldom exceeds five. The hypodermal tissue is partly a covering tissue; it normally assumes this function in certain plants and may assume it in others if the epidermis is injured. Hypodermal cells, as seen in transverse section, are usually angled and possess uniformly thickened walls. In longitudinal sections and in powdered materials, these cells appear rather long and similar to fibers, except for the fact that their end walls are blunt or square.

Cortical Parenchyma.—The primary cortex is bounded on the outside by the hypodermis and on the inside by the endodermis. The cortical cells represent the original parenchymatic tissue of the periblem region, and undergo but little change during the growth of the root. The cortex is usually several layers of cells in thickness; and within these cells much of the nutrient material of the plant is stored.

The individual cells are thin-walled and irregularly circular in outline, when seen in the transverse section.

Viewed in longitudinal section these cortical cells often appear rectangular or polygonal. In powdered materials the parenchymatic tissues may occur as rectangular, polygonal or irregularly circular cells, usually in mass and containing cell contents.

Endodermis.—This tissue separates the periblem tissues from those of the plerom, and is the innermost of the primary periblem tissues. The presence of endodermal tissues in a mature root is an indication of primary structure. The endodermis is very seldom more than one layer of cells in thickness and may be readily distinguished from the adjacent cellular elements. The individual endodermal cells are usually slightly colored and rectangular or polygonal in outline when viewed in transverse section. The cell walls may be uniformly thickened, or may show thickening on all sides except that toward the cortex. Thickening of these cell walls is due to deposition of suberin. In longitudinal sections or in powdered materials, the endodermal cells appear as rather long structures, resembling fibers. They differ from the latter in that one wall is thicker than the other and the end walls are blunt or square.

Phloem Bundles.—These tissues are developed in the parenchyma of the plerom zone and therefore are located within the endodermis. The phloem bundles occur as isolated groups of cells in the plerom parenchyma and usually alternate with the xylem bundles. Each phloem bundle consists of a transporting element (sieve cells) and a mechanical or supporting element (bast fibers). In transverse sec-

tions the sieve elements appear as groups of small very thin-walled cells in which the sieve plates or perforated end-walls may occasionally be apparent. Each group of sieve cells is surrounded by bast fibers, which, upon transverse section, appear as thick-walled angled cells with a rather small cavity or lumen. The true character of sieve cells can only be seen in longitudinal sections where they are apparent as long, thin-walled structures showing sieve plates at each end. The bast fibers on longitudinal view appear as long, thick-walled cells tapering toward each end.

Xylem Bundles.—These tissues are developed in the original plerom parenchyma in a manner similar to those of the phloem bundles. They are usually placed nearer the center of the plerom zone than are the phloem bundles with which they alternate. Each xylem bundle consists of transporting elements, ducts or vessels, and mechanical or supporting elements, wood or xylem fibers. In transverse section the ducts appear as large, irregularly circular, thick-walled cells, surrounded by smaller, angled, thick-walled wood fiber cells. In longitudinal view the ducts appear as continuous structures, the walls of which show markings varying according to the type of vessel. Wood fibers in longitudinal view appear as thick-walled cells, many times longer than broad and tapering toward each end.

The arrangement of fibro-vascular bundles in a circle within the endodermis is common to both primary and secondary root structures. The alternation of phloem bundles with xylem bundles, each remaining incomplete, is characteristic of the radial

type of bundle, found exclusively in roots of primary structure. It must be noted that the concentric and closed collateral types of fibro-vascular bundles are present in the primary roots of monocotyledons, so that while radial bundles indicate primary structure, not all primary roots possess radial bundles.

In powdered materials the ducts, wood fibers and bast fibers are always seen in longitudinal view. Sieve cells, being very delicate structures, are usually disintegrated during powdering and therefore are rarely apparent. The differentiation of wood fibers from bast fibers in a powdered sample is usually difficult and sometimes impossible. As bast fibers are generally longer and stronger than wood fibers, they are less likely to be broken during grinding. The lumen of a bast fiber is usually smaller than that of a wood fiber. The pores in fibers may appear as pits, or as slits extending diagonally in the wall of the fiber.

Pith Parenchyma.—This tissue represents the original tissue of the plerom zone. The individual cells are rather similar to the parenchyma cells of the cortex but are apt to be separated by larger intercellular spaces and to possess porous walls. The pith is in the center of the plerom region and is gradually replaced by xylem as the latter tissue increases. In monocotyledonous roots possessing bundles of the concentric and closed collateral types, there is no regular arrangement of fibro-vascular tissues, and the bundles are scattered within a central cylinder or stele. The *stele* is bounded by the endodermis and consists of the original parenchyma cells of the plerom zone.

SECONDARY ROOT STRUCTURE

Secondary root structures are present in mature dicotyledonous plants. They represent the permanent tissues of this class of plants and are replacements of, or enlargements upon, the primary tissues previously described. The changes occurring in the transition from primary to secondary structure may be summarized as follows:

1. The primary epidermis and hypodermis are replaced by tissues originating from a phellogen or bark cambium which develops within the primary cortex.
2. The endodermis is gradually replaced by tissues developed from the cambium.
3. The incomplete primary bundles are completed by the addition of xylem tissues on the inner face of the phloem bundles and phloem tissues on the outer face of each xylem bundle. These additions to the fibro-vascular bundles are due to the activity of the cambium, a meristematic tissue developed adjacent to the primary bundles.
4. Appearance of distinct medullary rays between the fibro-vascular bundles.
5. Replacement of the pith parenchyma by xylem tissues.

As shown in Plates 61, 62, the tissues usually present in roots of secondary structure and the order in which they occur, beginning at the outermost layer, are as follows:

1. Cork,
2. Phellogen,
3. Cortical parenchyma,
4. Phloem tissues,
5. Cambium,
6. Xylem tissues,
7. Medullary rays.

CHARACTERS OF THE SECONDARY ROOT TISSUES

Cork.—The corky tissues which replace the primary epidermis usually consist of several layers of
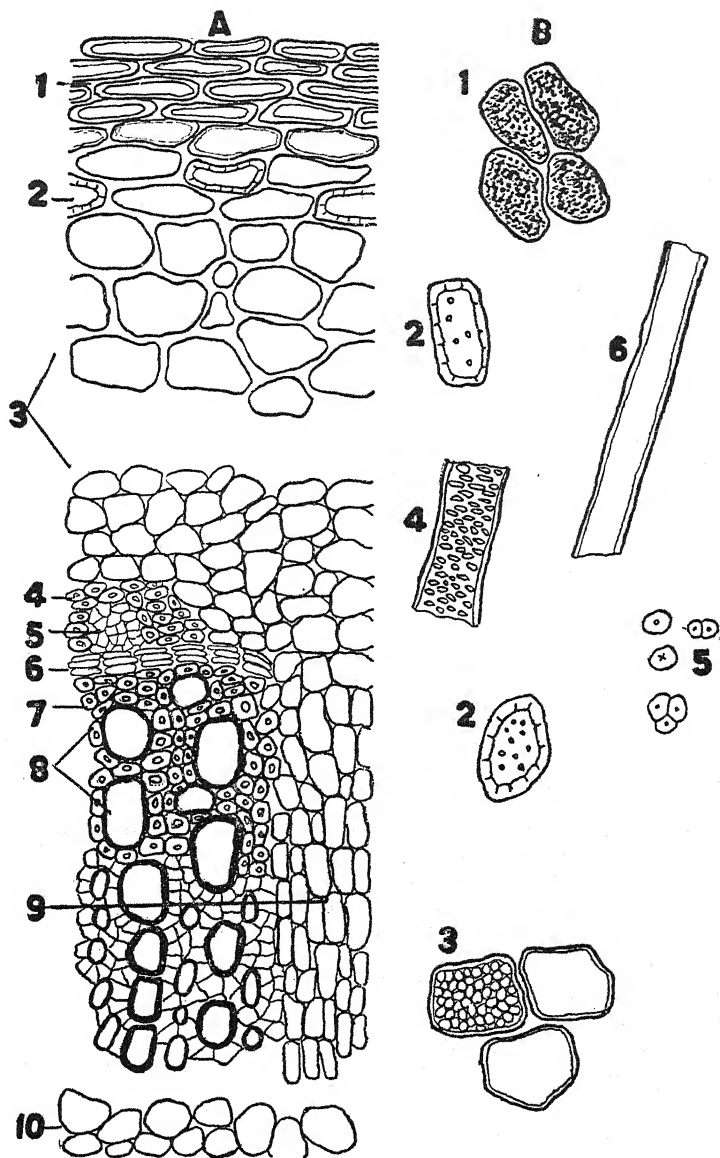
PLATE 61.—Secondary Root Structure.

A. Transverse Section, Aconite Root. 1. Cork. 2. Stone cells. 3. Cortical parenchyma. 4. Bast fibers. 5. Sieve cells. 6. Cambium. 7. Wood fibers. 8. Ducts. 9. Medullary ray. 10. Pith parenchyma (traces).

B. Powdered Aconite Root. 1. Cork. 2. Stone cells. 3. Parenchyma cells filled with starch. 4. Duct. 5. Starch grains. 6. Fiber.

dark colored cells. The individual cells are thin-walled, and may contain tannin deposits. In transverse sections cork cells appear rectangular or polygonal in outline. In surface view they are irregular in form and, because of their dark color and close contact, rarely present a clear and definite outline. In powdered materials cork cells always appear in surface view but, owing to the thickness of the fragments definite details of cell structure are seldom apparent.

Phellogen.—This meristematic tissue, although always present, is not easily distinguished from the cork cells and cortical parenchyma. It occupies a narrow zone immediately beneath the cork. The individual cells are rectangular in sectional view and brownish in color. As this tissue undergoes disintegration during grinding, it is never apparent in powdered materials.

Cortical Parenchyma.—This parenchymatic tissue is similar in every respect to the cells of the primary cortex. Occasionally sclerenchymatic tissues or stone cells are developed in the midst of the cortical cells of the root, to which they afford additional support and strength. In certain plants latex tubes ramify through the cells of this region.

Phloem and Xylem Bundles.—These elements are similar in structure to those already described under Primary Root Tissues. It must be noted that the fibro-vascular bundles of secondary structure are complete and consist of both xylem and phloem tissues. The bundles present in secondary or mature root structure are either of the open collateral type or the bi-collateral type. In the former, the xylem elements are toward the center and are separated from the
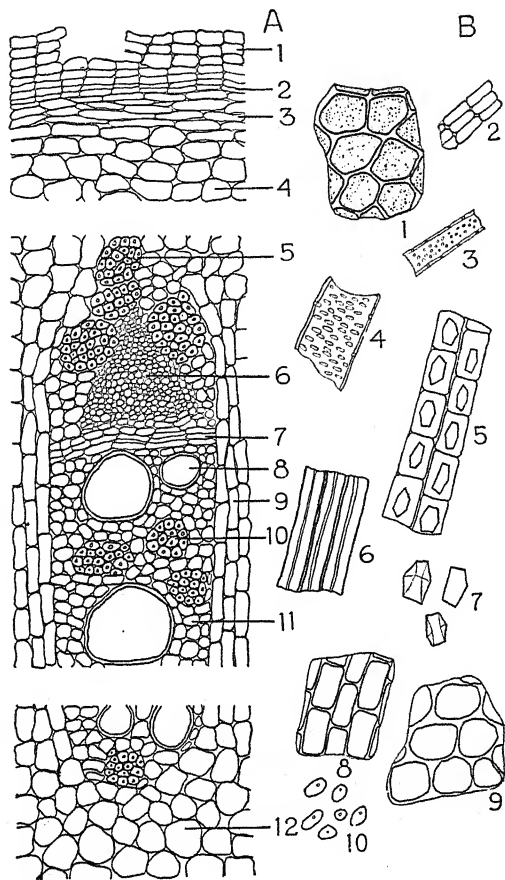
PLATE 62.—Secondary Root Structure.

A. Transverse Section of Licorice Root. 1. Cork. 2. Phellogen. 3. Phelloderm. 4. Cortical parenchyma. 5. Bast fibers. 6. Sieve tubes. 7. Cambium. 8. Ducts. 9. Medullary ray. 10. Wood fibers. 11. Wood parenchyma. 12. Remains of pith.
B. Powdered Licorice Root. 1. Cork, surface view. 2. Cork, sectional view. 3. Pitted vessel. 4. Reticulate vessel. 5. Crystal-bearing fibers. 6. Fiber mass. 7. Prismatic calcium oxalate. 8. Cortical parenchyma, longitudinal view. 9. Cortical parenchyma, transverse view. 10. Starch grains.

phloem by a strip or circle of cambium. In the bi-collateral bundle the xylem tissue bears cambium layers on its inner and outer surfaces, and each of these in turn gives rise to a phloem group.

Cambium.—This tissue is meristematic, possessing the power of cell division or reproduction, and is placed between the xylem and phloem elements. The individual cells, as seen in transverse section, appear as light-colored, thin-walled, rectangular, tangentially elongated cells. The cambium zone comprises one or more layers of cells, and although first existing in the form of detached strips attached to the primary bundles, soon forms a complete ring or circle in the root. In this manner the cambium not only bisects the bundles but also crosses the medullary rays or strips of tissue between the fibro-vascular bundles.

Medullary Rays.—The original medullary rays are wedge-shaped strips of plerom parenchyma between the fibro-vascular bundles. At first these strips are broad; but, owing to the development of new fibro-vascular bundles within them, they ultimately appear as narrow strips of tissue extending from the center of the root to the cortical region. The rays may be from one to five cells in width and as many as twelve cells in height. Seen on transverse section (Plate 70), the ray cells are long and rectangular with slightly thickened, porous or non-porous walls. On radial section (Plate 71A), they appear as groups of regularly arranged rectangular cells extending at right angles to the other tissues. On tangential section the rays appear as elliptical or oval patches, each of which contains several ray cells (Plate 71B). The dimensions of a medullary ray can only be determined by

examining sections made in different directions. Transverse sections show the width and length, radial sections the height, and tangential sections the width and height of the medullary rays.

CELL CONTENTS OF ROOTS

The cell contents of roots are usually stored in the parenchymatic cells. The stored materials may include most, if not all the substances mentioned in the section of cell contents. The most important contents from the histological standpoint are starch, inulin, calcium oxalate crystals and cystoliths.

FUNCTIONS OF ROOT TISSUES

The functions of the different structures present in roots may be tabulated as follows:

Covering tissues...... { Primary epidermis, Cork, Hypodermis.

Supporting tissues.... { Bast fibers, Wood fibers, Stone cells, Endodermis.

Absorbing tissues........Root hairs.

Conducting tissues.... { Sieve tubes, Ducts, Medullary rays, Latex tubes.

Assimilation tissues... { Leucoplasts in parenchyma cells, Parenchyma.

Storage tissues....... { Parenchyma, Latex tubes.

Meristematic tissues... { Phellogen, Cambium.

CHAPTER XIV

STEM STRUCTURE

PLANT stems may or may not undergo the series of changes incidental to the formation of secondary tissues. The stems and rhizomes of monocotyledonous plants usually retain the primary structures, with but slight variation, throughout life. In dicotyledons the primary structures undergo the changes consequent to the formation of secondary tissues. Herbaceous or annual stems exhibit primary structure. They last but a single season, although their rhizomes or roots may be biennial or perennial, giving origin to a new stem each season. The woody stems of most dicotyledonous plants are the result of the development of secondary structures.

PRIMARY STEM STRUCTURES

Primary stem structures are present in the earlier stages of stem development in both monocotyledons and dicotyledons. The dermatogen, periblem and plerom zones, to which reference has been made in the section dealing with the origin of tissues, and again in the section on root structures, are also present in the rudimentary stem. The primary tissues developed from these zones are in many respects similar to the primary tissues of roots; but several striking differences are apparent. The dermatogen

zone of stems gives rise to a primary epidermis, but
does not form tissues corresponding to those of the
root cap and root hairs. The primary epidermis is
strongly cutinized, may possess stomata and may
give origin to plant hairs or trichomes. The periblem
zone of stems gives rise to a hypodermis and an endo-
dermis, between which are several layers of parenchyma
cells, constituting the primary cortex. The hypo-
dermis of stems usually contains chlorophyll or green
coloring material. The walls of the primary cortical
cells may become thickened through deposition of
cellulose and assume collenchymatous forms. The
endodermis of stems is not as well developed as that
of the root. Fibro-vascular bundles are produced in
the plerom zone; but these bundles are complete in
form and consist of xylem, cambium and phloem
elements even in the earlier stages. The pith paren-
chyma, which in roots is gradually replaced by woody
tissues, persists in stems even throughout secondary
structure (Plate 65). The tissues usually present
in stems and rhizomes of primary structure, and the
order in which they occur, beginning with the outer-
most, are as follows:

1. Epidermis,
2. Hypodermis,
3. Cortical parenchyma,
4. Endodermis,
5. Phloem elements,
6. Cambium,
7. Xylem elements,
8. Pith parenchyma.

By reference to Plate 63 it will be noted that each
of these tissues presents structural characteristics
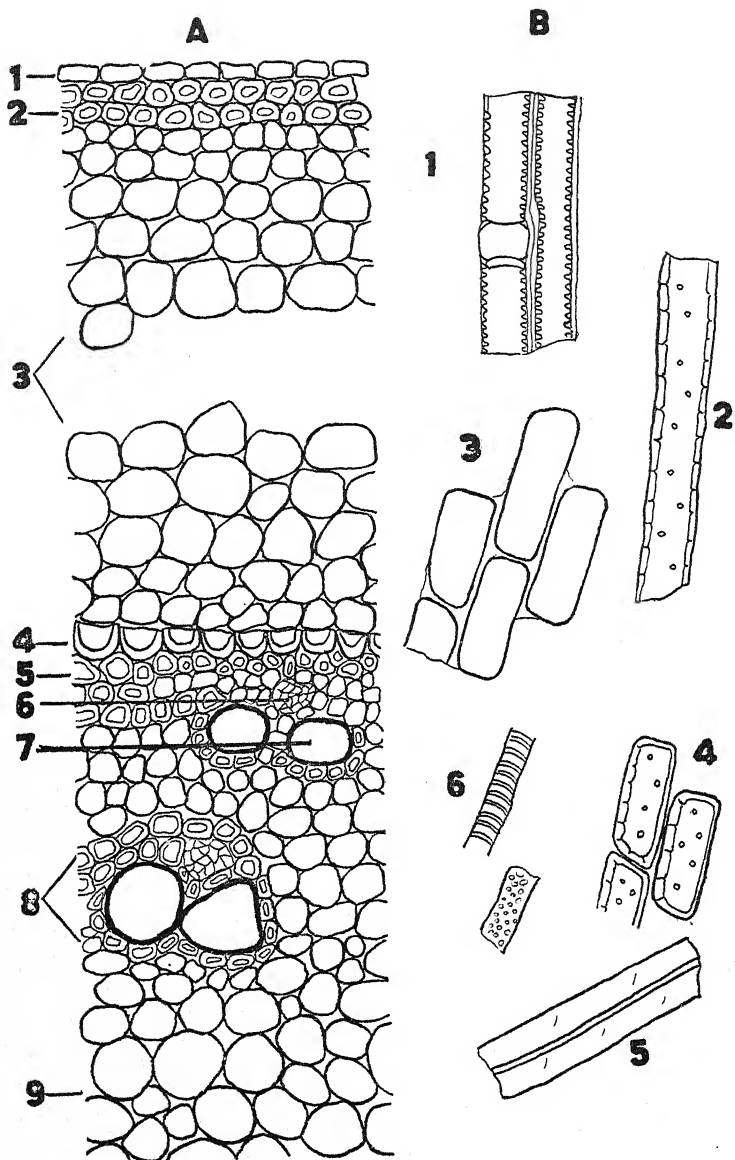
PLATE. 63.—Primary Stem Structure (Monocotyledonous).

A. Transverse Section, Triticum Rhizome. 1. Epidermis. 2. Hypodermis.
3. Cortical parenchyma. 4. Endodermis. 5. Fibers, surrounding sieve and ducts.
6. Sieve. 7. Ducts. 8. Collateral fibro-vascular bundle. 9. Pith parenchyma.
B. Powdered Triticum Rhizome. 1. Epidermis. 2. Hypodermis. 3. Parenchyma, longitudinal view. 4. Endodermis. 5. Fibers. 6. Vessels.

whereby it may be distinguished. Presence of primary tissues is an indication that the material is from an herbaceous or a monocotyledonous stem. Herbaceous stems may be readily distinguished from those of mature monocotyledons by the open collateral fibro-vascular bundles occurring in the specimen.

CHARACTERS OF THE PRIMARY STEM TISSUES

Epidermis.—The epidermis of herbaceous stems is a single layer of colorless transparent cells, the walls of which are impregnated with cutin. The individual cells, as seen upon the transverse section, are more or less rectangular and fit together very closely. Upon surface view the cells appear rectangular, polygonal or irregular in form. The epidermal cells of many plants show wavy or undulating walls, the irregularities of which fit into the walls of surrounding cells. Trichomes and stomata are often present and may be of assistance in identification. In powdered materials the epidermal cells are usually apparent on surface view and are frequently attached to the deeper tissues. In monocotyledonous stems of mature growth the epidermal tissues are so modified as to form a heavy covering several layers of cells in thickness. This, although differing from the cork layers of secondary stem structure, is very tough and resistant.

Hypodermis.—The hypodermal tissues of primary stems consist of several layers of thick-walled angled cells. In herbaceous stems, the hypodermal cells which contain chlorophyll are partly concerned in the production of starch. In monocotyledonous and

many herbaceous stems the hypodermis persists and forms a covering tissue which reinforces the epidermis.

Cortical Parenchyma.—The primary cortex of stems is bounded on the outside by the hypodermis and on the inside by the endodermis. The cortical cells represent the original parenchymatic tissues of the periblem region. The primary cortex is several layers of cells in thickness, and various nutrients may be stored within these cells. The individual cells are usually thin-walled and irregularly circular in outline when seen in transverse section. In longitudinal view the cortical cells are either rectangular or polygonal. In certain herbaceous stems, the cortical cells become thick-walled through deposition of extra amounts of cellulose upon their walls, and are thus transformed into collenchyma. These groups of collenchymatic cells serve as supporting tissues which, because of the short life of such plants, could not otherwise be produced in sufficient amounts. Stone cells and secreting cells are occasionally developed among the cortical cells. In certain plants latex tubes will occur in this cortical region.

Endodermis.—In stems, as well as in roots, this tissue separates the periblem tissues from those of the plerom. It is the innermost of the primary periblem tissue and its presence is an indication of primary stem structure. The endodermis usually consists of a single layer of cells and may not be as distinct as the corresponding tissue in roots. The individual cells are usually slightly colored and rectangular or polygonal in outline when viewed in transverse section. In certain stems the endodermal cell walls show uniform thickening on all sides; in other instances

the side of the cell toward the cortex remains thin-walled.

In longitudinal section the endodermal cells resemble the corresponding cells of the root, being long and similar to fibers except that one wall may be thicker than the other and that the end walls are blunt or square.

Phloem, Cambium and Xylem Tissues.—These tissues are developed as complete fibro-vascular bundles in the plerom zone and thus differ from the corresponding bundles in primary roots. In dicotyledonous stems the primary fibro-vascular bundles are of the open collateral type, or rarely the bi-collateral. In monocotyledonous stems the bundles are of the concentric, or more rarely, the closed collateral type. The cells entering into these different types of fibro-vascular bundles are similar in character to those described in the section dealing with secondary bundles in the root. (Chapter XIII.)

Pith Parenchyma.—This tissue represents the original parenchymatic tissue of the plerom zone. The individual cells possess slightly thickened and porous walls, are polygonal to spherical in form, and show exceedingly large intercellular spaces. The intercellular spaces become larger toward the center of the stem; therefore the central pith is a very loose structure, and may be lost or destroyed in the sectioning of herbaceous stems. In monocotyledonous stems the entire region within the endodermis is termed the *stele*, and consists of a groundwork of pith parenchyma cells in which are scattered isolated and irregularly arranged concentric or closed collateral bundles.

CELL CONTENTS OF PRIMARY STEMS

The cell contents of primary stems are stored in the parenchyma cells of the cortical and pith regions. Although these contents may include a variety of substances, the most important from the histological standpoint are chlorophyll grains, starch, calcium oxalate crystals and volatile oil globules.

FUNCTIONS OF PRIMARY STEM TISSUES

The functions of the different structures present in primary stems may be summarized as follows:

Covering tissues......... { Primary epidermis, Trichomes, Hypodermis.

Supporting tissues........ { Bast fibers, Wood fibers, Stone cells, Collenchyma, Endodermis.

Absorbing tissues...........Stomata.

Assmiliating and synthesis tissues........ { Chlorophyll in hypoderm layer, Secreting cells, Glandular hairs.

Conducting tissues........ { Sieve tubes, Ducts, Porous parenchyma, Latex tubes.

Storage tissues........... { Cortical parenchyma, Pith parenchyma, Latex tubes.

Meristematic tissues........Cambium.

SECONDARY STEM STRUCTURES

Secondary stem structures are present in mature dicotyledonous plants.   They represent the permanent tissues of this class of plants and are found in woody
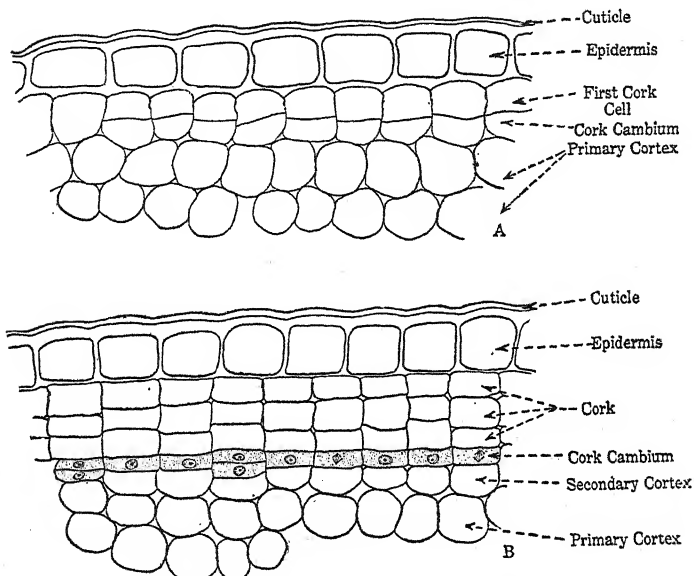
PLATE 64.—Cross-sections of portions of a stem showing origin of cork cambium (*A*), and the development of cork and secondary cortex from cork cambium (*B*).

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stems.   The changes occurring in the transition from primary to secondary structure may be summarized in the following statements:

1. The epidermal and hypodermal tissues are replaced by elements originating from a phellogen or bark cambium, which develops within the primary cortex.
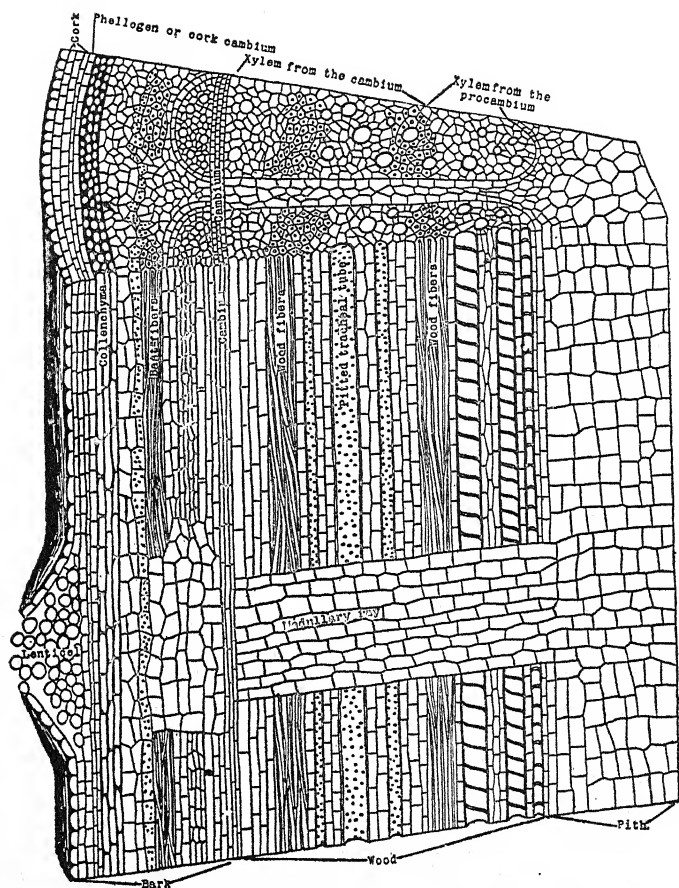
PLATE 65.—Diagram Showing the Topography of a Dicotyledonous Stem in which Additions of Tissues (Secondary Growth) Have Been Made through the Activity of Vascular Cambium and Cork Cambium. (After Stevens.)

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2. The endodermis is replaced by tissues developed from the cambium.
3. The original complete fibro-vascular bundles increase in size through additions of tissues by the cambium, and new bundles are formed in the broad primary medullary rays.
4. The cambium, originally in the form of arcs or segments between the xylem and phloem bundles, extends laterally and forms a complete ring or circle.
5. The pith is greatly reduced in size through extension of xylem elements and the formation of secondary bundles.

Owing to the thickness and complexity of the bark structures formed by the phellogen, it is more convenient to consider these separately from the woody tissues originating from the cambium. However, it must be kept in mind that both bark and wood are parts of the secondar ystem (Plate 65).

BARK STRUCTURE

From the histological standpoint, the bark of a secondary stem includes all structures external to the cambium. According to this statement it will be apparent that the phloem elements are considered with the bark tissues, rather than with the woody structures. True bark is only present in mature dicotyledonous plants, although monocotyledons possess a thickened epidermis which closely approximates bark in function and appearance.

As shown in Plates 66 and 67, the tissues usually present in barks, in the order of their arrangement, beginning with the outermost, are as follows:

1. Cork,
2. Phellogen and phelloderm,
3. Cortical parenchyma,
4. Stone cells,
5. Medullary rays,
6. Phloem elements.

It is convenient to divide the bark into outer, middle and inner layers or portions. In such a division, the outer bark includes the cork, phellogen and phelloderm structures. The middle bark is the region between the phelloderm and the outer ends of the medullary rays, and thus includes most of the cortical parenchyma and stone cells. The inner bark layer is traversed by the medullary rays and includes these structures, together with the phloem elements.

CHARACTER OF BARK STRUCTURES

Cork.—The periderm or corky layer of barks is usually very thick and consists chiefly of dead cells. The individual cells are generally dark colored, and, in transverse section, appear as rectangular or polygonal cells with thick or thin walls. The walls of cork cells are strongly suberized; and the intercellular spaces are so small that, except for the openings caused by the formation of lenticels, the cork forms an impervious covering around the stem tissues.

Phellogen and Phelloderm.—In many barks these layers may be so reduced as almost to escape notice. The phellogen consists of one or more layers of colorless, thin-walled, rectangular cells, immediately beneath the corky tissue. The walls of these cells are of cellulose and may be readily distinguished from the suberized cork cells by micro-chemical tests. The phelloderm consists of one or more layers of rather thick-walled cells located beneath the phellogen. These cells are irregular in form and may be similar to collenchyma in appearance. The phellogen always produces more cork than phelloderm, and the latter tissue may occasionally be reduced to a single layer
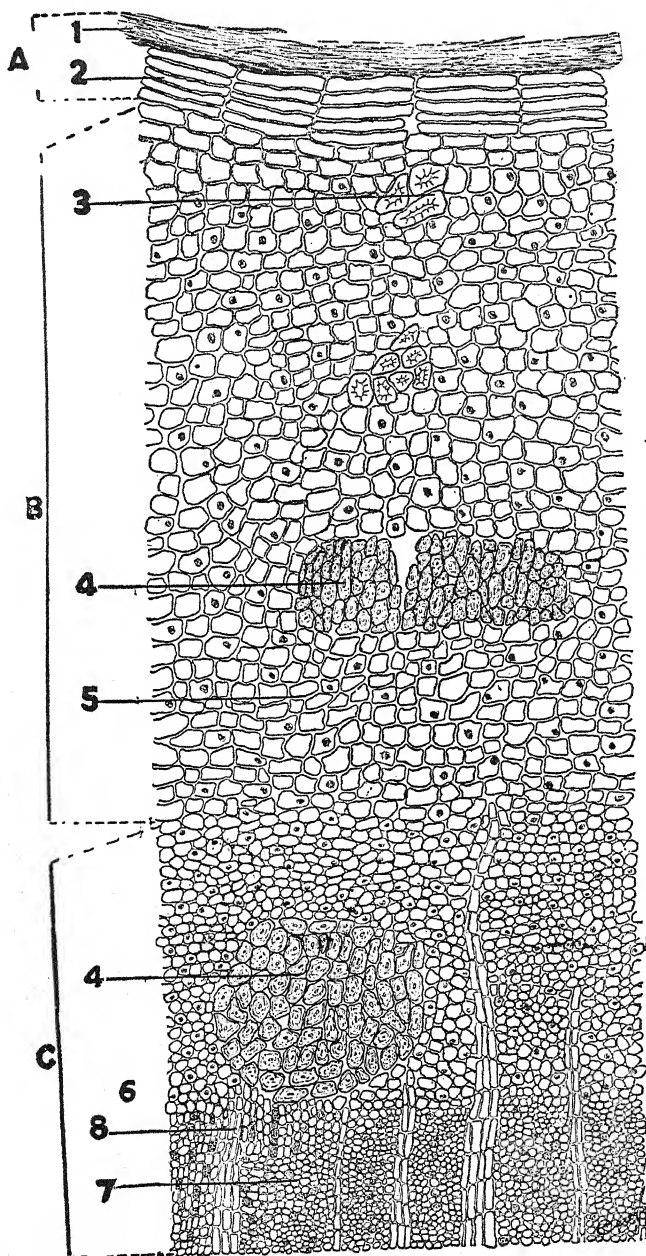
PLATE 66.—Bark Structure (Root).

Transverse Section, Chionanthus root bark. *A.* Outer bark. *B.* Middlebark. *C.* Inner bark. 1. Cork or periderm. 2. Phelloderm. 3. Stone cells. 4. Fibers. 4. Fibers. 5. Bark parenchyma cells (starch and crystals have been partially removed in preparation of the specimen). 6. Parenchyma. 7. Sieve tissue. 8. Medullary ray.

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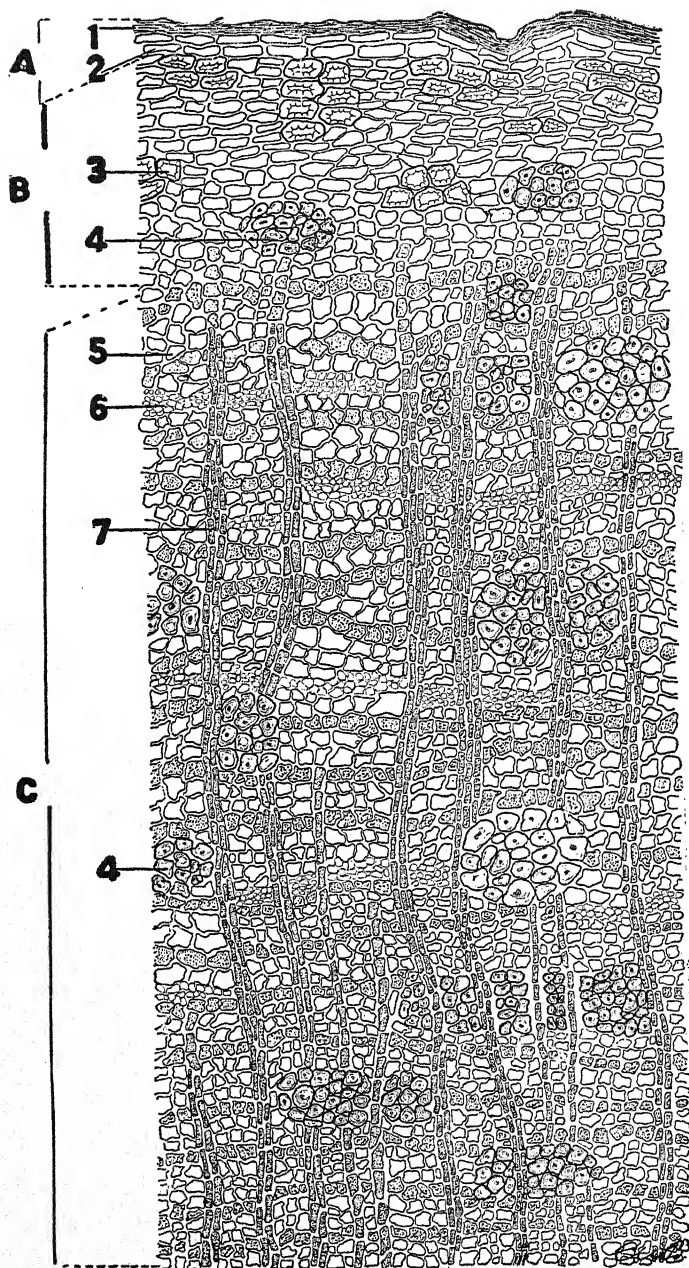
PLATE 67.—Bark Structure (Stem).

Transverse Section, Chionanthus stem bark. *A.* Outer bark. 1. Periderm. 2. Phelloderm. *B.* Middle bark. 3. Stone cells. 4. Fibers. *C.* Inner bark. 5. Bark parenchyma. 6. Sieve tissue. 7. Medullary ray. 4. Fibers.

of cells. In powdered materials cork cells are usually apparent on surface view, and occur in thick masses of dark color in which definite cell structure is visible
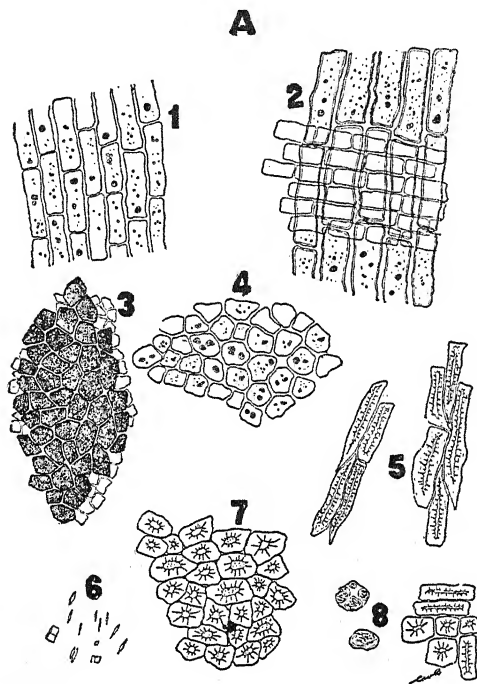
PLATE 68.—Bark Structure (Root).

A. Chionanthus, root bark. 1. Bark parenchyma (longitudinal view), containing starch, crystals and resin masses. 2. Bark parenchyma crossed by medullary ray cells. 3. Cork tissue. 4. Bark parenchyma (transverse view). 5. Fibers. 6. Crystals. 7. Stone cells. 8. Resin masses.

only with difficulty (Plates 68, 69). The phellogen and phelloderm tissues are never apparent in powdered materials.

Cortical Parenchyma.—The parenchymatic tissues of the middle bark vary greatly both in quantity and

in details of cell structure. Stone cells are rather
frequently found among the cortical cells, and secre-
tion cavities, when present, occur in this portion of
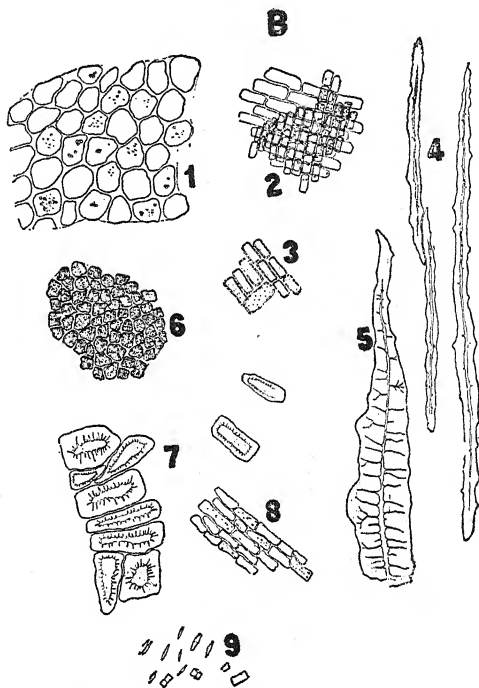
PLATE 69.—Bark Structure (Stem).

B. Chionanthus, stem bark. 1. Bark parenchyma (transverse view), contain-
ing starch, crystals and resin masses. 2. Bark parenchyma crossed by medullary
ray cells. 3. Medullary ray cells. 4. Fibers. 5. Fibers showing branching pores.
6. Cork tissue. 7. Stone cells. 8. Bark parenchyma (longitudinal view). 9. Crys-
tals.

the bark. The cortical cells contain starch, crystals
or other cell contents. This tissue is present in
powdered materials in the form of fragments con-
sisting of several thin-walled angled or circular cells
(Plates 68, 69).

Medullary Rays.—The ray cells present in barks are usually smaller than those within the cambium. Seen in transverse section, the individual cells are thin-walled, square or oblong in form, and each ray is made up of from one to four rows of cells. In tangential sections the ends of the rays are apparent as oval or elliptical groups of polygonal cells. In radial sections one obtains a side view of the rays and they appear as broad bands of rectangular cells extending at right angles to the surrounding tissues. In powdered materials medullary rays are generally seen on radial view (Plates 68, 69), and are usually in combination with parenchyma or other surrounding tissues. Occasionally, starch, crystals or other cell contents occur within the ray cells.

Phloem Elements.—The phloem elements in barks are usually located in the vicinity of the medullary ray cells. The bast fibers occur as groups of cells between the medullary rays or toward the outer ends of these. In transverse sections the individual fibers appear as small, thick-walled cells, readily distinguished from the surrounding elements, with the possible exception of stone cells. The lumen of these fiber cells is often so reduced as to appear as a mere dot or mark in the center of the cell. Pores traversing the wall of the fiber are rarely apparent in sectional views. The sieve elements in barks are usually collapsed, and appear as very small, irregular, thin-walled cells adjacent to the bast fibers. In powdered materials the bast fibers are usually prominent, whereas sieve elements are rarely apparent. The fibers occur singly and in masses, frequently combined with parenchyma or ray tissues (Plates 68, 69).

CELL CONTENTS

The cell contents in barks are stored in the cortical parenchyma, medullary rays and occasionally within stone cells. Resins and volatile oils are contained in special secretion cells or cavities. The stored materials include starch, crystals, tannins, resins, gums and volatile oils.

WOOD STRUCTURE

From a histological standpoint wood is the material remaining after removal of the bark. According to this statement, the wood includes all structures internal to the cambium. True wood is never present in the monocotyledonous stems which retain their primary structures through life. The structures usually present in woods, in the order of their arrangement, beginning with the outermost, are as follows:

1. Xylem elements,
2. Medullary rays,
3. Pith parenchyma (traces).

CHARACTER OF WOOD STRUCTURES

Xylem Elements.—The predominant structures in woody stems are wood fibers and ducts. A lignified type of parenchyma may be associated with these xylem elements. The wood fibers immediately adjacent to the cambium are living cells; but those nearer the center of the stem are to all intents lifeless. Each fiber is a typical prosenchymatic structure and, in longitudinal section, appears as a long, thick-walled cell tapering toward each end. The ends of fiber cells

are usually pointed, and interlock or fit into corresponding ends of fibers below and above. Occasionally the ends of a fiber are branched. In transverse section, the fibers appear as angled cells with fairly thick walls and small central cavities (Plate 70). Pores penetrating the fiber wall may be apparent upon longitudinal view, but are rarely seen in transverse sections of a fiber. The ducts of woody stems are of the pitted or finely reticulate types. In transverse sections they appear as large, thick-walled cavities or cells, frequently with porous walls (Plate 70). The ducts usually occur in groups and are surrounded by wood fibers or woody parenchyma. It is in longitudinal section that the true nature of the duct is apparent, as its length and the character of its walls can only be seen in this view. Wood fibers and ducts extend parallel to each other and both are at right angles to the medullary rays. Woody parenchyma differs from fibrous tissue in that the cells are much shorter and thinner-walled (Plate 71). In form the woody parenchyma cells appear similar to true parenchyma, but the cellulose walls of the latter have become partially lignified. In powdered woods (Plate 70) the fibers and ducts are always apparent on longitudinal view. Owing to the tenacity with which they adhere, wood fibers in powdered materials usually occur in masses. The cavities of the fibers occasionally contain crystals, coloring materials, volatile oils and rarely starch grains.

Medullary Rays.—These structures extend from the central region of the stem, through the xylem elements, to the cambium and thence to the bark tissues. They provide channels for the lateral trans-

portation of nutrients, and communicate with both ducts and sieve tubes. The ray cells occur in groups which, viewed in different aspects, show great differences in appearance. In transverse sections the rays occur as bands composed of one to four rows of rather thin-walled, nearly rectangular cells (Plate 70). On this view the width of the entire ray and the width and length of the individual cells may be ascertained. In radial section the rays appear as broad bands composed of several rows of thin-walled, rectangular cells (Plate 71A). The height of the entire ray and the height and length of the ray cells are apparent in this view. In tangential sections the rays occur as oval or elliptical groups of circular or polygonal cells (Plate 71B). The width and height of the individual cells of the entire ray are apparent in this view. Medullary ray cells may or may not possess porous walls. The ray cells may contain starch, crystals or other cell contents. In powdered materials the ray cells (Plate 70) are apparent in radial and occasionally in transverse view. The masses of wood fibers frequently show attached ray cells, and in such fragments the ray cells are at right angles to the fibers.

Pith Parenchyma.—In mature woody stems this element is much reduced in quantity and may be entirely obscured. In other instances its position is indicated by a hollow cavity or space at the center of the stem. The individual cells are irregularly circular with thin and frequently porous walls. Owing to the small amount of pith parenchyma present in a mature woody stem, this element is rarely apparent in powdered materials.
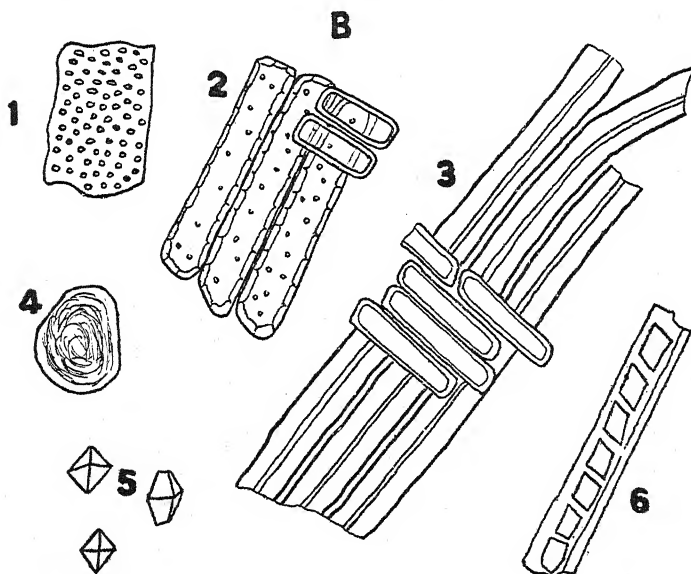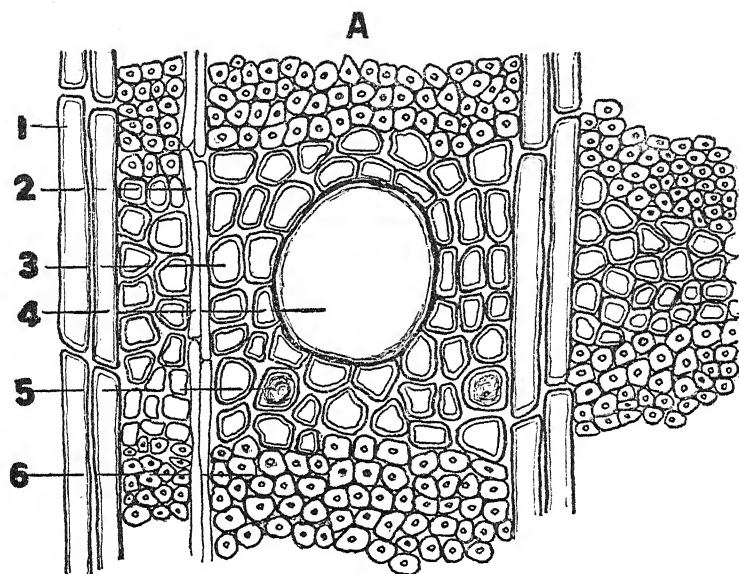
PLATE 70.—Wood Structure.

A. Transverse Section, Hematoxylon. 1. Medullary rays. 2. Resin passages.
3. Woody parenchyma. 4. Duct. 5. Resin mass in parenchyma cell. 6. Wood
fibers.
B. Powdered Hematoxylon. 1. Vessel, pitted type. 2. Woody parenchyma
with fragment of ray. 3. Fibers crossed by medullary ray cells. 4. Resin mass.
5. Crystals, prismatic type. 6. Crystal-bearing fiber
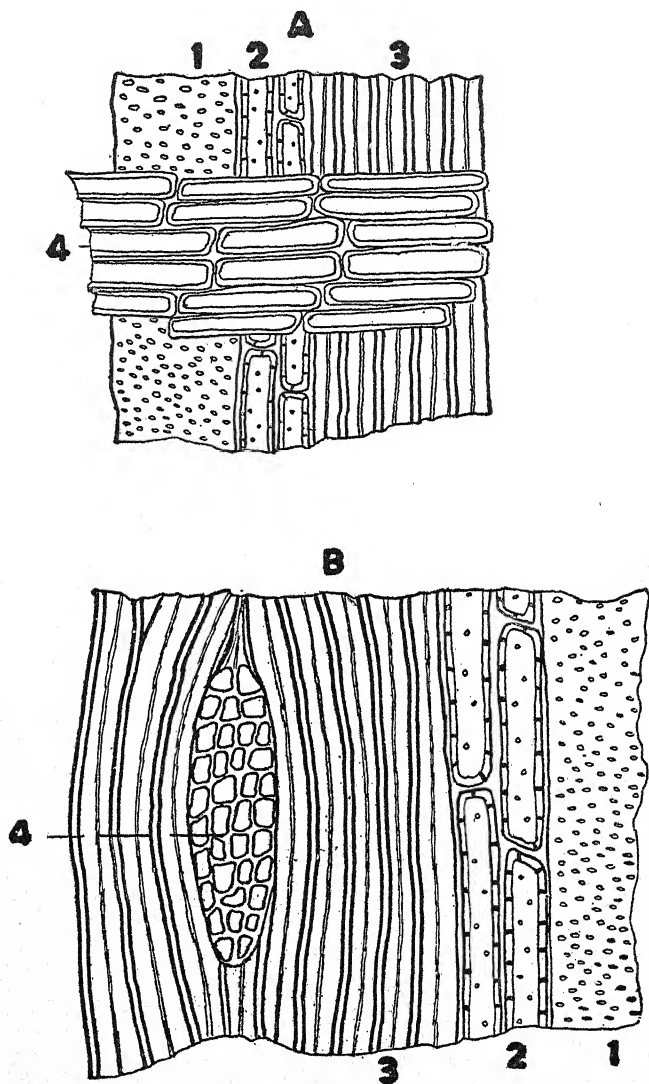
PLATE 71.—Wood Structure.

A. Radial Section, Hematoxylon. 1. Duct. 2. Woody parenchyma. 3. Fibers. 4. Medullary ray cells.
B. Tangential Section, Hematoxylon. 1. Duct. 2. Woody parenchyma. 3. Fibers. 4. Medullary ray cells.

CELL CONTENTS

The cell contents of woods are stored in the cavities of the fibers, in the ray cells and in the pith parenchyma cells. The stored materials include crystals, resins and volatile oils. Starch is rarely present in woods.

FUNCTIONS OF SECONDARY STEM TISSUES

The functions of the different structures present in secondary stems may be summarized as follows:

Covering tissues.......... { Cork or periderm, Phelloderm.

Supporting tissues........ { Bast fibers, Wood fibers, Stone cells.

Absorbing tissues...........Lenticels.

Conducting tissues........ { Sieve tubes, Ducts, Medullary rays, Latex tubes.

Assimilating and
    synthesis tissues..........Secretion cells.

Storage tissues........... { Bark parenchyma, Latex tubes.

Meristematic tissues...... { Phellogen, Cambium.

RHIZOMES

The characters and arrangements of tissues in underground stems or rhizomes correspond closely to those of overground stems. Monocotyledonous rhizomes retain the primary structures through life while those of the dicotyledonous class undergo the

changes incidental to the formation of secondary tissues. Rhizomes showing primary structure are readily distinguished from roots by the presence of nodes or joints, and by the absence of root hairs. They are further distinguished from roots by the possession of complete fibro-vascular bundles even in the earlier stages of growth.

CHAPTER XV

LEAF STRUCTURE

THE leaves are lateral extensions of the stem, which have been expanded or otherwise modified in structure to function as manufacturing and assimilating organs. In their histological characters, leaves show many of the elements characteristic of primary stem structure; and, although these elements are more or less modified, a direct relationship can be traced between them and the cortex and central cylinder of the stem. Leaves manufacture the greater portion of the starch and may take part in the manufacture of other plant nutrients. These nutrient materials are transported through the leaf stem or petiole, and thence through the vascular tissues of the stem, to plant organs requiring nutrients or to storage tissues. The tissues present in leaves, in the order of their arrangement are as follows:

1. Upper epidermis,
2. Upper palisade,
3. Leaf parenchyma or mesophyll,
4. Fibro-vascular elements,
5. Lower palisade (occasionally),
6. Lower epidermis.

CHARACTERS OF THE LEAF TISSUES

Epidermis.—The leaf epidermis usually consists of a single layer of strongly cutinized cells which

function as a transparent, protective tissue. On transverse section (Plates 72, 73), the epidermal membrane will be found to consist of rectangular or
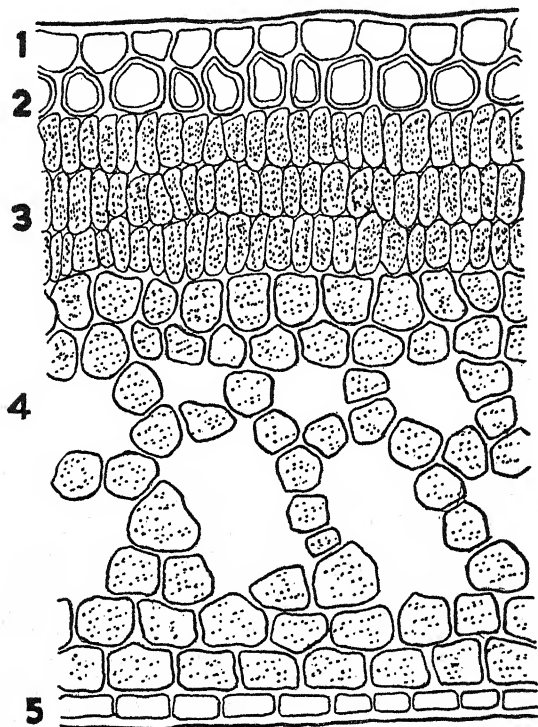
PLATE 72.—Leaf Structure.

Transverse Section, Oleander Leaf. 1. Upper epidermis. 2. Subepidermal or hypodermal layer. 3. Palisade cells. 4. Mesophyll or leaf parenchyma. 5. Lower epidermis.

polygonal, thin-walled, colorless cells fitting tightly together with practically no intercellular spaces. The exposed wall of the epidermal cell may show more or less thickening, or may be further modified to form glandular or non-glandular trichomes. Occasionally

a subepidermal or hypodermal layer of cells (Plate 73, No. 2) occurs immediately beneath the epidermis.
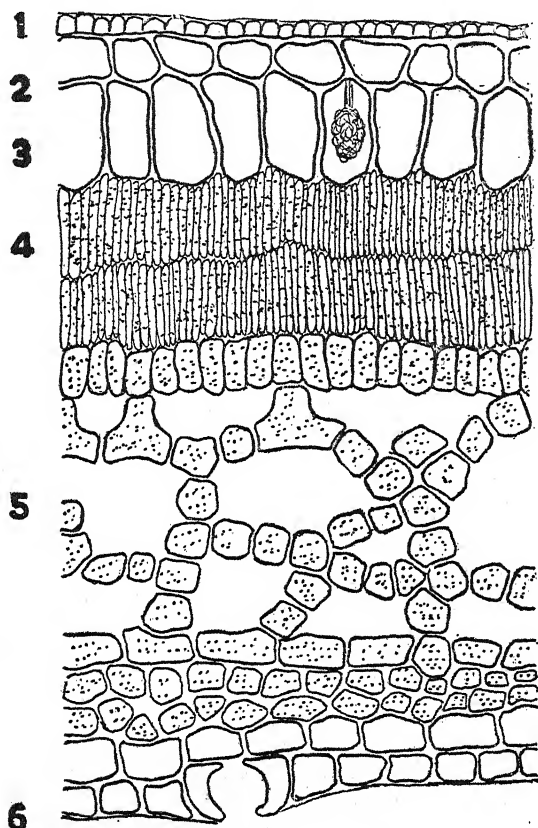
PLATE 73.—Leaf Structure.

Transverse Section, Ficus Leaf. 1. Upper epidermis. 2. Subepidermal or hypodermal layer. 3. Cystolith in subepidermal cell. 4. Palisade cells. 5. Mesophyll or leaf parenchyma. 6. Lower epidermis, showing stoma.

These subepidermal cells are frequently present in the leaves of tropical plants and may be of use in preventing excessive evaporation of water from the inner

leaf tissues.   Stomata or breathing pores are present in the lower epidermis in the majority of leaves, and, in a few instances, also occur in the upper epidermis. On surface view the epidermal cells of different plants show definite characteristics as regards form of cells, character of their walls, location of stomata and occurrence of trichomes.   On surface view the epidermal cells may be rectangular, polygonal or irregular in form (Plate 37).   Their walls may be thin, irregularly thickened or beaded, and may show striations extending parallel with the border of the cell.   The stomata may be located on the same level as the epidermal cells, or may be above or below the latter. Trichomes, both glandular and non-glandular, are outgrowths of the epidermal cells, and are exceedingly important in the identification of leaves in powdered condition.   The more common forms of trichomes are described in connection with the covering tissues in Chapter VII.   In powdered materials the epidermal cells are usually apparent on surface view, although they may be so adherent to the deeper tissues that details of cellular structure can only be seen with difficulty.

Palisade Cells.—These cells are located immediately below the epidermal or subepidermal tissues and are arranged in one or more layers (Plates 72, 73).   Palisade layers may occur on both surfaces of the leaf or may be present only on the upper surface. *Unifacial leaves* are those possessing palisade tissues on both surfaces, while *bifacial leaves* possess only an upper palisade layer.   Occasionally palisade tissues are lacking on both surfaces of the leaf.   The individual palisade cells appear as narrow rectangles with

the short side toward the epidermis. The walls are thin, and the cells are filled with chloroplasts. This tissue is active in the photosynthesis of starch. The palisade cells may be so grouped that several appear to be in contact with one of the leaf parenchyma cells and the latter cells may function as *collecting* or *funnel cells* to receive the soluble starch produced in the palisade tissue. Oil or resin cells occasionally occur in the palisade layers. On surface view the palisade cells may be seen through the epidermis, and appear in small polygonal forms within the larger epidermal cells. In powdered materials the palisade cells may be seen in transverse section or on surface view. The green masses frequently apparent in powdered leaves consist of epidermal, palisade and perhaps leaf parenchyma cells, which hold together during powdering, and which are so thick that the cellular structure of each element in the mass is obscured.

Leaf Parenchyma.—The mesophyll or leaf parenchyma elements represent the original tissues of the leaf, or those which correspond to the tissues present in the plerom zone of the stem. The mesophyll consists of several layers of irregularly circular, thin-walled cells, loosely connected and showing large intercellular spaces (Plates 72, 73). This tissue fills in the space between the upper and lower palisade layers, or is the tissue between the epidermal layers when the palisade is lacking. The cells contain chloroplasts, and function partly as tissues for the manufacture of starch and partly as tissues for the transportation of the assimilation starch produced in the palisade cells. Stone cells of the branched

type are occasionally present in the mesophyll layer. Crystals may occur in the leaf parenchyma cells, and starch grains in process of formation may be demonstrated by special methods. In powdered materials the leaf parenchyma is usually present in the form of green masses consisting either of mesophyll cells or consolidations of these with palisade and epidermis (Plate 74). Owing to the thickness of these fragments, details of cellular structure are apt to be obscured.

Fibro-vascular Tissues.—The fibro-vascular elements or veins of the leaf ramify in the mesophyll layer and correspond both in development and structure to the bundles of the stem. In dicotyledonous leaves the bundles are of the collateral type, while in monocotyledons the concentric and closed collateral arrangements may prevail. As in other parts of the plant, the fibro-vascular bundles function as supporting and conducting tissues. The work of transportation, or conduction of crude materials and manufactured nutrients to and from the leaf, requires a large and widespread vascular system; therefore the conducting elements of the bundles are more developed than are the supporting elements. The fibro-vascular tissue in the leaf stem, or petiole, is continued into the midvein in pinnately veined leaves, or into the primary veins in leaves of palmate venation. The midvein or primary veins branch repeatedly and form innumerable small veins, thereby connecting with every part of the leaf. As the fibrous elements of the bundles are often comparatively small in amount, collenchymatic tissues may be developed in the vicinity of the larger veins, thus affording additional support. The fibro-vascular bundles are often surrounded by a sheath (*starch*

sheath) which corresponds to the rudimentary endo-dermis and which may function as a conducting element. The fibro-vascular bundles of the petiole and the sheaths surrounding them communicate either directly or indirectly, with bundles in the parent stem. In
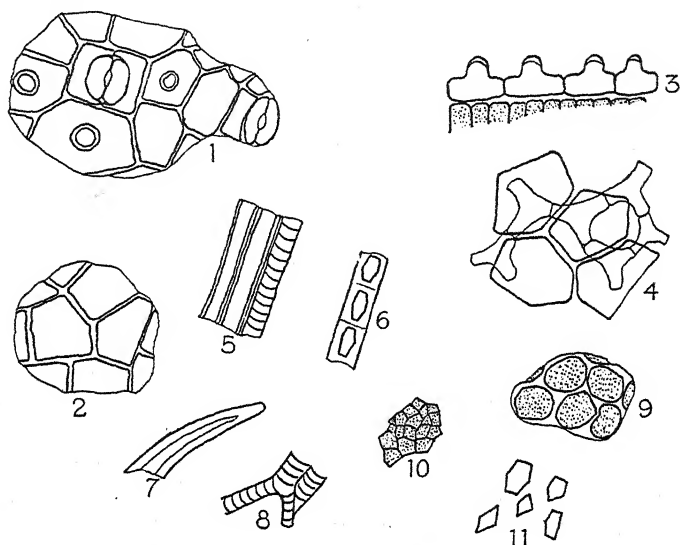
PLATE 74.—Leaf Structure (Powdered).

Coca Leaf. 1. Lower epidermis with stomata and papillae. 2. Upper epidermis. 3. Lower epidermis in sectional view showing papillae and a portion of palisade. 4. Lower epidermis with irregular, spongy mesophyll cells attached. 5. Fibrovascular tissues. 6. Crystal-bearing fiber. 7. Unicellular trichome from Inga flower. 8. Spiral vessels. 9. Mesophyll parenchyma. 10. Palisade cells in surface view. 11. Prismatic calcium oxalate.

powdered materials the fibers and vessels are usually clearly apparent. The fibers occur in groups, are thin-walled and comparatively few in number. The vessels occur either separately or in conjunction with the fiber masses. Spiral or annular vessels are the predominating types. In powdered leaves all of these elements are apparent on longitudinal view.

CELL CONTENTS

The cell contents of leaves are stored within the palisade cells, mesophyll parenchyma, secretion cells and glandular hairs, and occasionally in the fibers. The most important stored material is chlorophyll a protoplasmic cell content. The nonprotoplasmic contents include volatile oils, calcium oxalate crystals and calcium carbonate deposits or cystoliths.

FUNCTIONS OF LEAF TISSUES

The functions of the different structures present in leaves may be tabulated as follows:

Covering tissues.......... { Epidermis, Trichomes.

Supporting tissues........ { Bast fibers (traces), Wood fibers (traces), Collenchyma, Stone cells (rarely).

Conducting tissues........ { Sieve tubes, Ducts, Vessel sheaths, Mesophyll.

Absorbing tissues...........Stomata.

Assimilating and synthesis tissues........ { Palisade, Mesophyll, Secretion cells, Glandular hairs.

Storage tissues........... { Secretion cells, Glandular hairs.

CHAPTER XVI

FLOWER STRUCTURE

THE floral organs are developed from leaves, which, by various processes of modification, have been fitted to perform the functions of producing seeds. In many instances the relationship between the floral part and the leaf, as regards both characters and structural peculiarities, is clearly evident. The sepals, or parts of the calyx, usually bear a close resemblance to leaves, in color and form. In other floral organs the modifications so obscure this relationship that the true nature of the organ in question can only be ascertained by considering its *ontogeny,* or the changes occurring during the period of its development. The stamens bear little resemblance to foliate leaves; and it is only by a study of the changes which occur during their development that the structural relationship has been established. Each organ of the flower possesses structural characters peculiar to itself, and more or less common to corresponding parts in other flowers. However, the deviations from typical characters are so frequent in the floral organs, that it is impossible to give descriptions which will apply in all instances. The structures present in a typical complete flower, beginning with those of the outermost circle, are as follows:

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1. Bract tissues,
2. Calyx tissues,
3. Corolla tissues,
4. Stamen tissues,
5. Pistil tissues,
6. Stem tissues.

CHARACTERS OF THE FLORAL TISSUES

Bract Tissues.—These structures, which are known collectively as the *epicalyx*, are not a part of the flower as they do not originate from the torus, or structure which gives rise to the floral organs. The bracts originate from the stem and are foliage leaves which, because of modification in form and contiguity of position, simulate floral parts. Their histological characters are similar to those of leaves, and they show epidermal tissues with stomata and perhaps trichomes, palisade layers, mesophyll and the fibro-vascular structures of leaves.

Calyx Tissues.—The sepals, or calyx divisions, are classed as floral organs because they originate from the torus. In most flowers the sepals are green and leaf-like; but in those flowers in which the corolla is wanting (monochlamydeous), the calyx may simulate a corolla in appearance and structure. The histological elements present in sepals include epidermal tissues, mesophyll and simple forms of fibro-vascular bundles. The epidermis (page 75) bears stomata on the outer or free surface and may also show trichomes. The green tissues consist of loosely arranged mesophyll or leaf parenchyma cells containing chlorophyll. The palisade layers are usually lacking. The fibro-vascular tissues are not as wide-
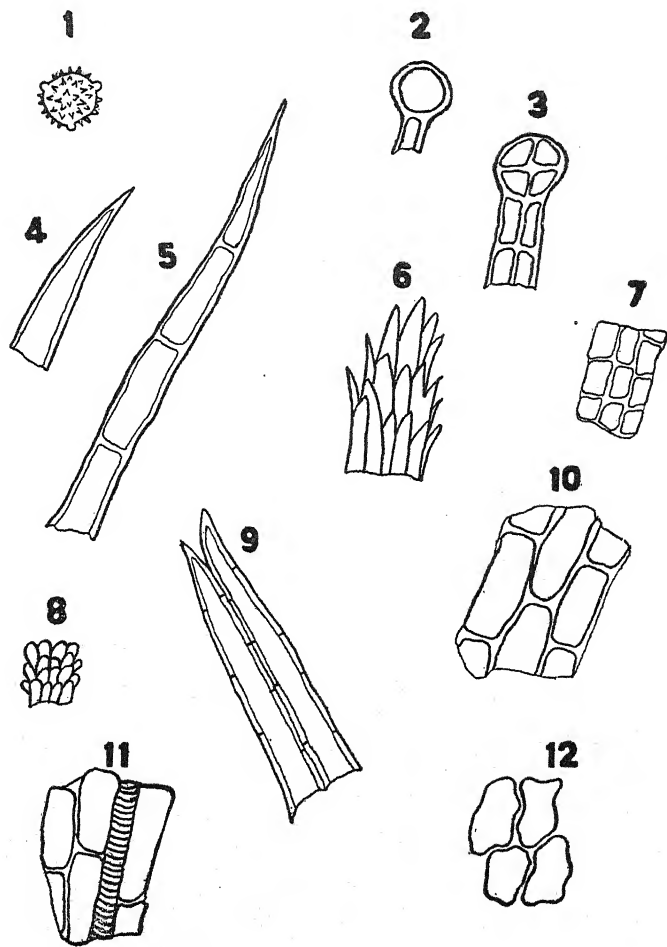
PLATE 75.—Flower Structure.

Powdered Arnica Flowers. 1. Pollen grain. 2. Glandular trichome (unicellular). 3. Glandular trichome (multicellular). 4. Nonglandular trichome (unicellular). 5. Nonglandular trichome (multicellular). 6. Nonglandular trichome (multiseriate). 7. Stamen tissue. 8. Stigma tissue, showing papillæ. 9. Nonglandular trichome (bicellular). 10. Petal tissue. 11. Petal tissue, showing spiral vessel. 12. Calyx tissue.

spread nor as complex as those of leaves; and the fibrous elements are much reduced in amount.

Corolla Tissues.—The *petals* or corolla divisions constitute the circle of floral leaves next inside the calyx. If there be but one circle of floral leaves in the flower it is classed as calyx, no matter what its color or form. The corolla in most flowers is white or variously colored, the color being due to pigments contained in the parenchyma cells or dissolved in the liquids within and around the cells. The histological elements present in petals include epidermal tissues, parenchyma and traces of fibro-vascular bundles. The epidermis (Plate 75), is rather similar to that of the leaf, rarely shows stomata and often possesses papillæ. Striated and beaded forms of cell walls occur in this tissue, and glandular hairs are occasionally present. The parenchyma corresponds to the meso-phyll of leaves and contains pigments or colored cell liquids, but no chlorophyll. The fibro-vascular tissues are even more rudimentary than those of the calyx and consist of annular, spiral or reticulate vessels, almost lacking in fibrous supporting elements. Glandular structures (*nectaries*) may arise from the epidermal surface of the petals. These nectaries are usually very simple secreting membranes and produce a sweet liquid which attracts insects and thus aids in pollination.

Stamen Tissues.—The *stamens* collectively constitute the *androecium* or male reproductive structures of the flower. Each stamen consists of anther, connective and filament. The anther consists of two parts (thecæ) attached to each other, and to the stem-like structure or filament, by the connective.

The histological elements present in stamens include epidermal tissues, parenchyma, fibro-vascular tissues and pollen grains. The epidermis covering the anther and filament is similar to that of the corolla. Parenchyma is present in the filament and connective, but is wanting or much reduced in the anther. The parenchyma cells (Plate 75), are small and are disposed around the simple, central fibro-vascular bundle of each filament. The fibro-vascular elements consist of spiral or annular vessels with practically no fibrous tissue. The *pollen* (Plate 75) is the most important histological element of the stamen tissues, for in many instances the grains present characters of great importance to the analyst. Pollen grains, although uniform in a given plant, show great variation in size, form and surface markings. They may be spherical, ellipsoidal, triangulate or polygonal in form. Pores, grooves and elevations may be apparent on the outer surface. In many instances the outer surface shows numerous spiny projections which are probably for the purpose of firmly fixing the grain upon the stigmatic surface. Grains possessing these spines or projections are termed *spinose* in contradistinction to the smooth-surfaced grains.

Pistil Tissues.—The *pistils* collectively constitute the *gynœcium* or female reproductive structures in the flower. Each pistil consists of stigma, style and ovary. The stigma is the uppermost portion of the pistil and is connected with the ovary by the style. The histological elements present in the pistil include epidermal tissues, parenchyma, fibro-vascular elements and ovules. The epidermal tissues of the stigma are of two distinct varieties. The upper surface of

the stigma or the surface designed as a resting place for the pollen, is usually roughened either by papillæ (Plate 75), or by a peculiar palisade formation of the epidermal cells. In other instances the stigmatic surface is smooth, but secretes viscid liquids which hold the pollen when the latter comes into contact with it. The lower or outer surface of the stigma is covered by a thin membranous epidermis, similar to that generally occurring in leaves. The style and ovary are covered with a thin epidermal tissue which may show stomata and trichomes. The parenchymatic tissues of stigma, style and ovary consist of loosely connected cells showing large intercellular spaces. These parenchyma cells usually contain chloroplasts. The fibro-vascular tissues are of great importance in the style and ovary, as in the former organ they often provide a channel for the descent of the pollen tube, and in the latter they conduct nutrients to the developing embryo. The ovules are covered with thin but resistant epidermal layers, within which are parenchyma cells containing large amounts of nutrient material and the developing embryo.

Stem Tissues.—The stem tissues of the flower are in every respect similar to other plant stems and exhibit the elements noted in the section on Primary Stems (Chapter XIV). Stone cells and fibers are of frequent occurrence in flower stems, and their presence often affords a means of detecting excess stems in flower powders.

CELL CONTENTS

The cell contents of flowers are stored within the parenchyma cells of the different floral organs. The

protoplasmic cell contents include chlorophyll and various other pigments. The non-protoplasmic contents include calcium oxalate crystals and the volatile oils secreted by glandular hairs. Starch is rarely present.

FUNCTIONS OF FLOWER TISSUES

The functions of the different tissues present in flowers may be summarized as follows:

Covering tissues......... { Epidermis,
  Trichomes.

Supporting tissues...... { Fibers,
  Stone cells (in stems),

Absorbing tissues.........Stomata.

Conducting tissues.........Ducts.

Assimilating and
  secretory tissues...... { Palisade of calyx,
  Glandular hairs,
  Secretion cells,
  Nectaries.

Reproducing tissues..... { Inner epidermal layers of anther
  and ovary.

CHAPTER XVII

FRUIT STRUCTURE

A FRUIT is a ripened pistil containing the fertilized ovules or seeds, together with modified structures derived from almost any part of the flower with the possible exceptions of stamens and stigma. The term *pericarp*, as applied to fruits, includes all structures excepting the seeds. The pericarp is divisible into three layers, an *exocarp* (*epicarp*) or outer layer, a *mesocarp* or middle layer and an *endocarp* or inner layer. In closely related classes of fruits, these layers present similar structural characters; but in different classes of fruits the structural variations are very great. It is impossible to cover all these variations by general statements; therefore but a few types, and more especially those of pharmaceutical interest, will be considered in this section. It must be borne in mind that even fruits of these classes will show more or less variation from the structures described. Fruits may be subdivided, according to the texture of the pericarp, into fleshy and dry types. While the fleshy fruits do not dehisce or open at maturity to permit the escape of seeds, the dry fruits are divisible into dehiscent and indehiscent subclasses.

CREMOCARPS

Fruits of the cremocarp class are of the dry, indehiscent type and are further characterized by the fact

that they separate at maturity into one-seeded parts. The fruits of the Umbelliferæ, which include anise, fennel, caraway, coriander and angelica, are termed cremocarps. In drug commerce these fruits are known as seeds; but this is incorrect, as upon close examination they will be found to consist of a pericarp within which is the seed (Plate 76).
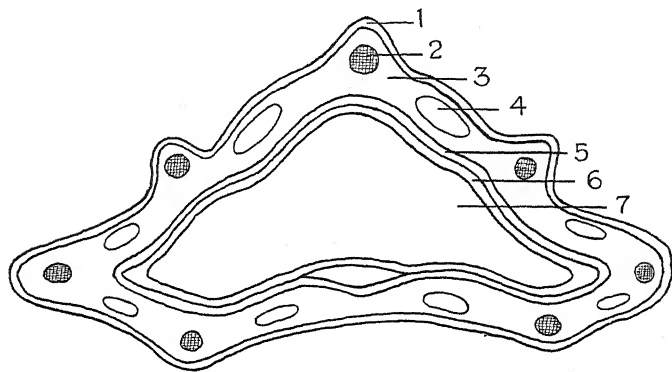
PLATE 76.—Fruit Structure (Cremocarp).

Transverse Section of Fennel Fruit, Diagrammatic. 1. Exocarp. 2. Fibrovascular bundles in mesocarp. 3. Mesocarp. 4. Vittæ. 5. Endocarp. 6. Seed coats. 7. Seed.

Exocarp Tissues.—As seen in transverse section, the exocarp in this type of fruits consists of a single layer of colored, rather thick-walled, irregularly elongated cells (Plate 77). These cells may possess striated walls, rarely show stomata, and may give rise to papillæ or unicellular trichomes. On surface view the exocarp appears to be composed of colored polygonal or irregular cells, and is usually adherent to the tissues of the mesocarp. Surface views are usually obtained in powdered materials although an occasional transverse fragment may be apparent.

Mesocarp Tissues.—The mesocarp layer consists of rather thick-walled parenchyma cells, oil ducts and fibro-vascular elements (Plate 77). This layer varies in thickness, owing to the presence of ribs or elevations which are apparent on the outer surface of the fruit. The number of ribs is fairly constant for each species of umbelliferous fruits. The oil ducts are called *vittæ* and are continuous structures, although they may show markings indicating the junctions of the cells composing them. They extend through the entire length of the fruit and are of the schizogenous type. The number of vittæ is constant for a given fruit, and each duct is surrounded by a layer of secreting cells outside of which is a layer of thickened parenchyma cells. The fibro-vascular bundles consist of small numbers of short, thick-walled fibers in conjunction with annular or spiral vessels. In powdered materials the parenchyma appears as masses of thick, white-walled cells, either separate from or combined with fragments of the vittæ. The vittæ are always seen on longitudinal or surface view, and appear as broad, colored bands traversing the parenchyma masses. The fibro-vascular elements are comparatively few, the fibers and vessels being apparent on longitudinal view.

Endocarp Tissues.—The endocarp usually consists of a single layer, or, at most, two layers, of irregularly rectangular cells (Plate 77). In powdered materials the endocarp cells are usually apparent on surface view as masses of thick-walled, long, rectangular cells, either separated from or attached to the mesocarp tissues. In certain fruits of this class the endocarp cells are arranged in groups, the cells of each group extending
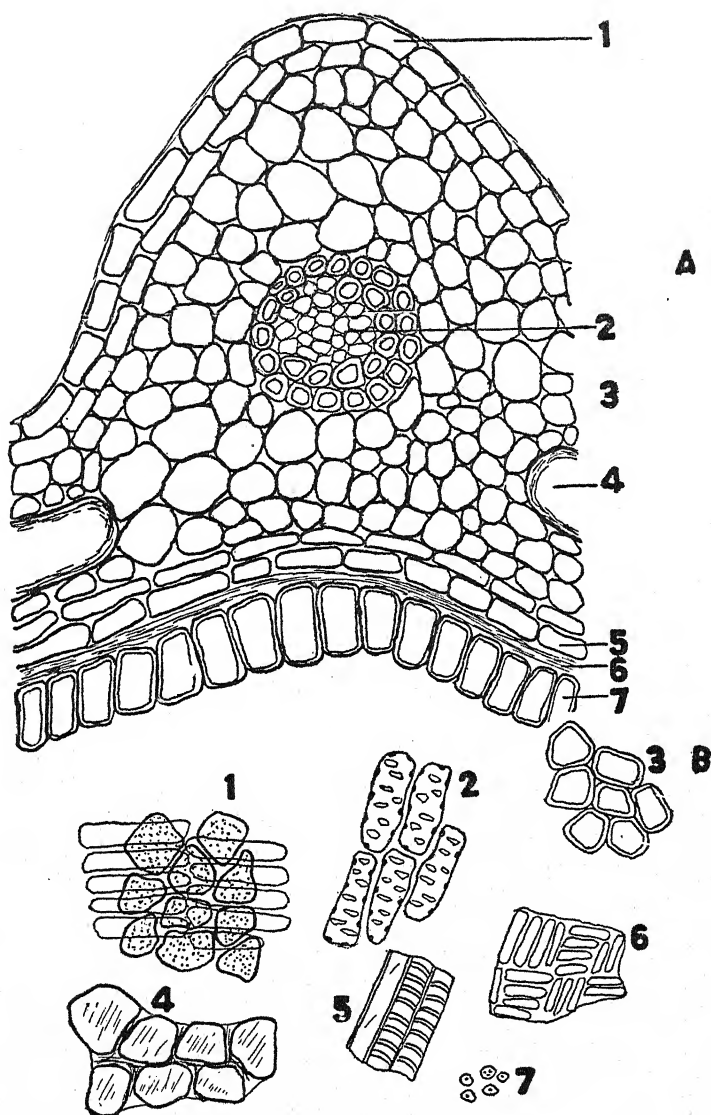
PLATE 77.—Fruit Structure.

A. Transverse Section, Fennel Fruit. 1. Epicarp or epidermis. 2. Fibro-vascular bundle. 3. Mesocarp parenchyma. 4. Secretion cavity or vitta. 5. Endocarp layers. 6. Seed coat layers. 7. Endosperm of seed parenchyma.

B. Powdered Fennel Fruit. 1. Epicarp adherent to dark cells lining vittæ. 2. Porous parenchyma of mesocarp. 3. Endosperm cells. 4. Parenchyma of mesocarp. 5. Fibro-vascular elements. 6. Endocarp cells. 7. Aleurone grains from seed parenchyma.

in different directions from those of the surrounding groups, in a manner similar to the strips in parquet flooring.

CAPSULE FRUITS

The fruits of this class (Plate 78), consist of two or more united carpels, and the fruits dehisce or open at maturity to permit the escape of seeds. They are non-fleshy, and dehiscence may occur by longitudinal splitting along the carpellary edges (septicidal), along the midrib of the carpel (loculicidal) or by pores at the apex of the fruit.

Exocarp Tissues.— These fruits are generally covered by a single layer of thickened, tangentially elongated cells. In surface preparations the exocarp cells of vanilla are beaded, and in the poppy fruit stomata are apparent.

Mesocarp Tissues.— A hypodermis of two or more

PLATE 78.—Fruit Structure (Capsule). Transverse Section of Tulip Fruit. 1. Exocarp of membranous cells with cuticle on surface. 2. Mesocarp region with rudimentary sieve cells. 3. Mesocarp region with rudimentary ducts. 4. Extent of mesocarp region. 5. Endocarp.

layers of tangentially elongated cells underlies the epidermis. This hypodermis is collenchymatic in nature and the cells may be porous. The parenchyma of the mesocarp is thin-walled and of loose texture in vanilla, but in the poppy capsule is thick-walled and compact. Cell contents of various kinds may be contained in these parenchyma cells, and fibro-vascular elements with spiral vessels occur. Latex tubes are present in this region in the poppy capsule.

Endocarp Tissues.—The endocarp consists of a single layer of tangentially elongated cells. The endocarp cells of vanilla are thin-walled and give rise to long secreting hairs which contain an aromatic resin. The endocarp cells of poppy are extremely thick-walled, and stomata may be scattered throughout this layer.

DRUPACEOUS FRUITS

The drupaceous fruits (Plates 79, 80) are fleshly indehiscent, and their seeds are separated from the mesocarp layer by a hard endocarp. These characters also apply to certain *aggregate* fruits, as the raspberry and blackberry, which are merely aggregations of small drupaceous fruits (Plate 81) attached to a torus or receptacle. These aggregate fruits result from the development of several separated pistils of a flower, each pistil forming a separate unit of the fruit. The drupelets cling together because of the proximity of the points of attachment of the several pistils on the torus. It must be borne in mind that the following statements are general and that, as previously noted, variations are common.

Exocarp Tissues.—This portion of a drupaceous fruit is usually a membrane consisting of a single layer of cells These cells are rectangular or tangentially elongated in sectional view, with the free surface often slightly thick-walled or showing a distinct cuticle. On surface view this epidermis appears in the form of angled cells, occasionally with beaded walls. Stomata and trichomes may also be apparent. The trichomes are responsible for the velvety surface of the peach and raspberry. Stomata do not occur as frequently as trichomes and are probably of little importance from the functional standpoint. In certain fruits the epidermal cells contain pigments responsible for the characteristic coloration.

Mesocarp Tissues.—In several fruits of this type a distinct hypodermal region of one or more layers of cells occurs in contact with the epidermis. This hypodermis serves as an additional covering tissue and consists of collenchyma or stone cells. A collenchymatized hypodermis is likely to be of non-porous cells. This tissue responds to the microchemical tests for cellulose. A sclerenchymatized hypodermis consists of stone cells, usually of the porous type, and these may be radially elongated. The hypodermal tissues usually adhere to those of the epidermis, and in powdered materials masses representing these two layers are of frequent occurrence. If the epidermis is not too deeply colored, the hypodermal cells will be visible through the former. If the hypodermis is collenchymatic in character the cells show less tendency to separate in milling than if it consists of stone cells.

The bulk of the mesocarp of a drupaceous fruit
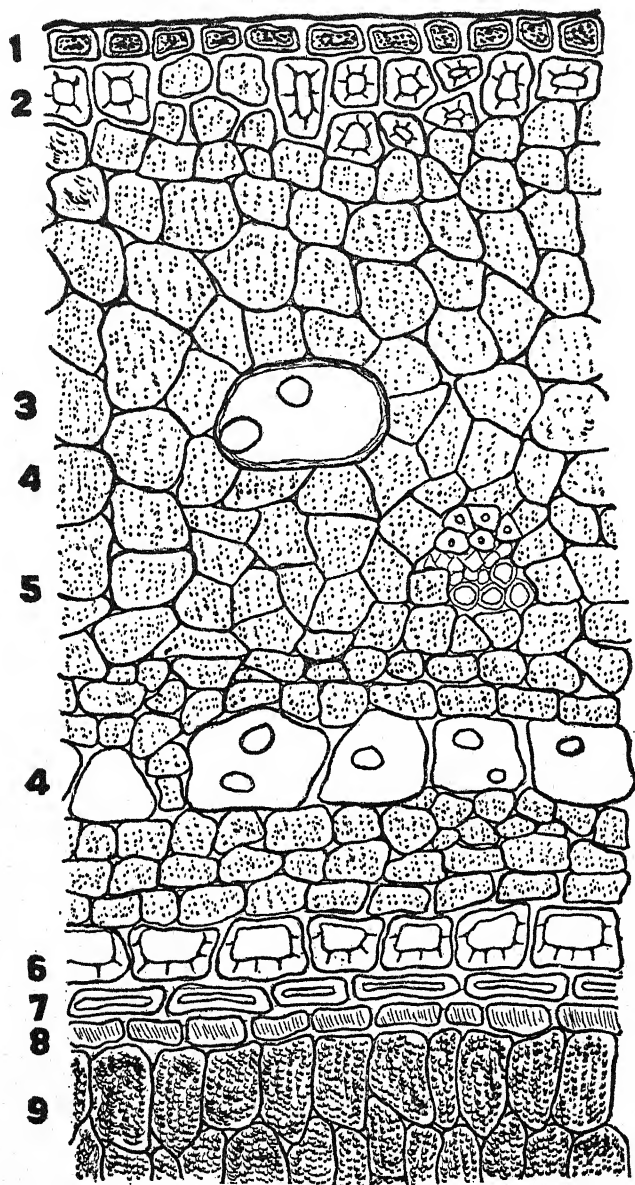
PLATE 79.—Fruit Structure.

Transverse Section, Black Pepper. 1. Epicarp layer. 2. Stone cells of hypodermal or outer mesocarp layer. 3. Mesocarp parenchyma. 4. Secretion cavity. 5. Fibro-vascular bundle. 6. Stone cells of endocarp or inner mesocarp layer. 7. Seed coat tissue, testa and tegmen. 8. Aleurone layer, surrounding the endosperm. 9. Endosperm or seed parenchyma.
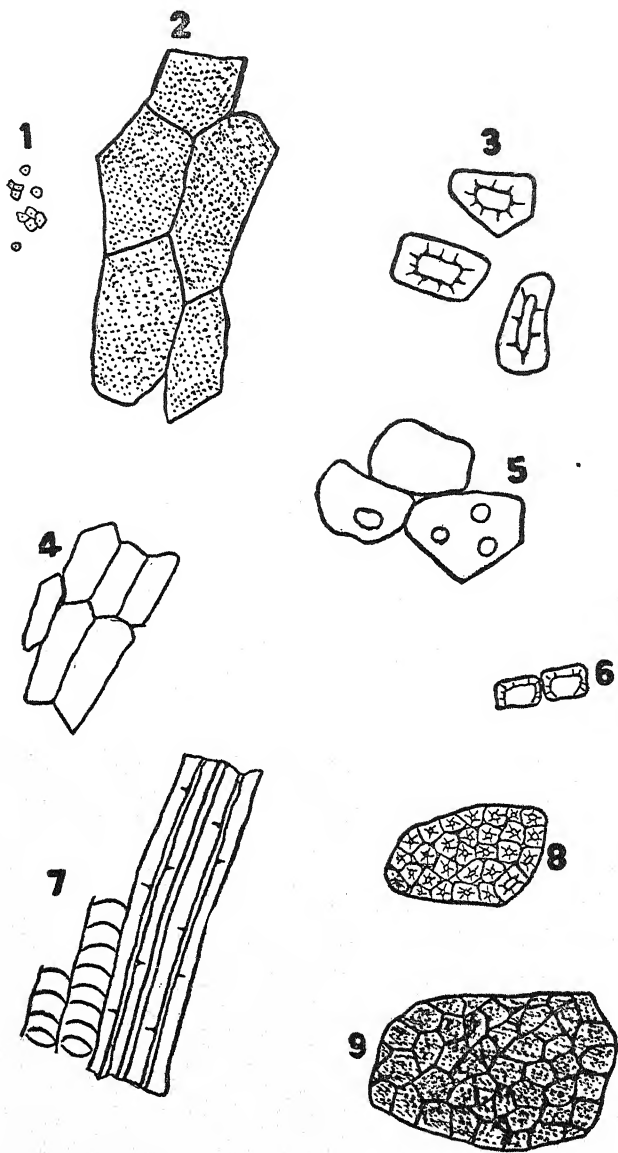
PLATE 80.—Fruit Structure.

Powdered Black Pepper. 1. Starch, single grains. 2. Starch, aggregates. 3. Stone cells from outer mesocarp layers. 4. Seed coat tissue. 5. Mesocarp parenchyma containing oil globules. 6. Endocarp stone cells, sectional view. 7. Fibrovascular elements. 8. Endocarp stone cells, surface view. 9. Epicarp tissue.

consists of thin-walled parenchyma cells of isodia-
metric and irregular forms.
Cell contents are stored in
these parenchyma cells; and,
if such contents are insoluble
in the cell liquids, or if desic-
cation has occurred, they are
of service in identification.
Starch, crystalline calcium
oxalate and oils may occur in
visible form in this portion of
the fruit. The sugars respon-
sible for the sweet taste of
many of our edible fruits can
only be recognized when the
material has been dried under
conditions favorable to the
crystallization of the material.
Secretion cells may occur in
the mesocarp region, and the
volatile oils and resins are
often apparent within the
cavities of such structures.
Occasionally stone cells, either
individually or in groups, are
interspersed between the par-
enchymatic elements of the
mesocarp. Fibro-vascular tis-
sues also occur in this region,
the amounts of these tissues
varying in the different fruits.
The amount of fibrous tissue is
much reduced and the vascu-
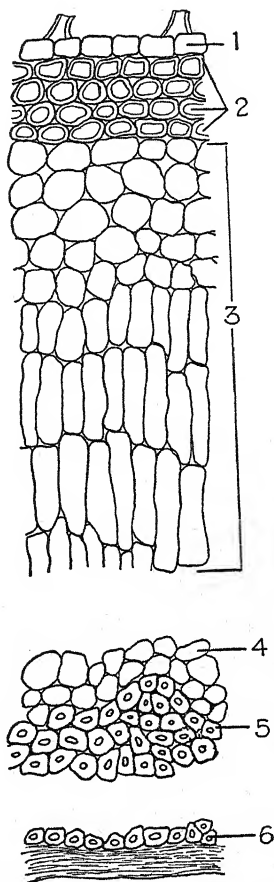
PLATE 81. — Fruit Structure
(Aggregate and Drupaceous).

Transverse Section of Raspberry
Drupelet. 1. Exocarp of membran-
ous cells with bases of trichomes. 2.
Mesocarp, outer layers of collenchy-
matic cells. 3. Mesocarp, middle
layers of large parenchyma cells. 4.
Mesocarp, inner layers of small par-
enchyma cells. 5. Endocarp of thick-
walled stone cells.

lar elements are usually vessels or ducts of the spiral and annular types. In powdered materials the parenchyma cells and secretory structures are usually much broken and the cell contents are scattered throughout the field. The fibro-vascular elements are always visible in longitudinal view in powdered fruits.

Endocarp Tissues.—The endocarp of this class of fruits consists invariably of stone cells with but little trace of other elements. In extent this tissue ranges from a single layer, as in pepper, to the large stone or putamen of the peach. These stone cells are usually of the porous type and their form ranges from isodiametric in the central portions of the endocarp to radially elongated in the outer and inner layers. Although the walls of these stone cells are for the most part uniformly thickened, in an endocarp consisting of but a single layer of cells the outer wall will frequently be thinner than the inner and radial walls. In powdered materials the stone cells of the endocarp tend to adhere in masses and are usually smaller than those derived from a hypodermal layer.

BACCATE FRUITS

The baccate or berry fruits are characterized by the occurrence of numerous seeds loosely embedded in the fleshy portion of the fruit and not contained within a hard endocarp, as in the drupaceous fruits. The endocarp may be so little differentiated from the the mesocarp as to be non-apparent, and in no instance does it approach the high degree of development found in fruits of the drupaceous type (Plate 82).

Exocarp Tissues.—This portion of the fruit is generally a single layer of epidermal cells and is very

similar to the corresponding region in the drupaceous fruits. Trichomes are rarely present, and, in addi-

tion to the thickened cuticle on the free surface of the epidermal cells, a waxy substance may be secreted. This coating of wax gives the grayish color to certain grapes and is readily rubbed off in handling. In powdered materials this portion of the fruit is similar in appearance to the corresponding portion of the drupaceous fruits.

Mesocarp Tissues.— A hypodermal region, consisting of one or more layers of cells, may be present beneath the epidermis as in the tomato; or the epidermis may be in direct contact with the thin-walled parenchyma of the fruit flesh, as in the grape. The bulk of this portion of the fruit is of thin-walled parenchyma containing large amounts of liquid. Fibro-

PLATE 82.—Fruit Structure (Baccate). Transverse Section of Allspice Fruit. 1. Exocarp. 2. Mesocarp. 3. Schizogenous oil cavity. 4. Stone cells. 5. Endocarp.

vascular tissues, with the fibrous portion much reduced and the vascular elements of spiral and annular vessels,

extend among the parenchyma cells. Upon drying and powdering, the parenchymatic elements of the mesocarp are likely to be so disintegrated as to lack characteristic appearance, and the vascular elements, apparent on longitudinal view, are the only tissues recognizable.

Endocarp Tissues.—This portion of the baccate fruits is the least uniform in character. In the tomato and grape there is practically no differentiation between the mesocarp parenchyma and the endocarp, and in the tomato the endocarp is rendered still more indistinguishable by a gelatinization of the cell walls. In the gooseberry the endocarp is parenchymatic and differs from the mesocarp parenchyma only in size. In the cranberry the endocarp consists of wavy-walled cells with beaded walls. Still more striking is the appearance of the stomata in this region. The blueberry shows scattered stone cells in the endocarp. In the currant the endocarp is completely sclerenchymatized and consists of a single layer of irregular, elongated stone cells. The only tissues possible of recognition in this fruit layer in powdered materials are the stone cells, if these be present.

MULTIPLE FRUITS

The fruits of this class are formed by the union of the ovaries of many flowers and are thus distinguished from the aggregate fruits, which result from the adhesion of many pistils of the same flower. This class is subdivided into (1) fleshy fruits composed of coalesced scale-like parts forming a *galbalus*, of which the juniper berry is an example; (2) fleshy fruits in which the units are contained within a re-

ceptacle forming the *syconium*, of which the fig is an example; (3) dry fruits composed of many overlapping scale-like units forming a *strobile*, of which the hop is an example.

It is difficult to formulate statements in regard to the structure of this type of fruits, because the term "multiple" is more or less general, and the units composing the different multiple fruits vary widely in character. The individual units of the fig are drupelets; those of hops, achenes; and in the juniper they are fleshy scales. In addition to these units or individuals in the multiple fruit, the structure of the accessory and accrescent parts must be taken into account. For this reason, each of the above subclasses will be described separately.

JUNIPER BERRY (Galbalus)

Each berry is formed through the union of three fleshy scales or bracts, traces of which can be seen at the apex of the fruit.

Exocarp.—This portion consists of a single layer of thick-walled and slightly beaded cells, appearing in tangentially elongated form in sectional view and is rounded to polygonal form in surface preparations. The free surface of these cells secretes a grayish waxy substance, and dark-colored contents are present.

Mesocarp.—The fruit pulp consists of thin-walled parenchyma, large schizogenous oil reservoirs, fibrovascular elements and small numbers of stone cells. A hypodermis, consisting of a few layers of collenchymatized cells, may be present in the portion in contact with the epidermis.

Endocarp.—A distinct layer of thick-walled small cells occurs only at the central portion of the fruit, and this corresponds to an endocarp region. Undifferentiated fruit pulp is in contact with the major portion of the seed.

FIG (Syconium)

The fleshy portion of this fruit is a receptacle formed from a hollow branch, the inner surface of which bears the small fruit units or drupelets, each with a minute stem.

Receptacle.—The outer surface is covered by small thick-walled epidermal cells, giving rise to unicellular trichomes. Beneath this epidermal layer is a hypodermis of partially collenchymatized cells occasionally containing small aggregates of calcium oxalate. The bulk of the receptacle consists of large parenchymatic cells which may contain crystallized sugars. Latex tubes and fibro-vascular tissues ramify among these parenchyma cells. The inner surface of the receptacle is covered by thin-walled small cells, which occasionally bear small unicellular trichomes.

Pericarp.—The small fruits borne on the interior of the receptacle are of the drupaceous type, with all the tissues present but in greatly reduced amounts.

HOPS (Strobile)

The units of this fruit are membranous bracts, and the fruitlets are small achenes borne at the base of each bract.

Bract.—This part of the fruit is leaf-like in structure, covered with wavy-walled epidermal cells bearing unicellular trichomes and with stomata apparent in the lower epidermis. The interior tissue consists of thin-walled parenchymatic cells containing chloroplasts and aggregate crystals of calcium oxalate. At least part of this inner parenchyma is of very loose texture, and fibro-vascular elements composed of spiral vessels and thin-walled fibers are present.

Pericarp (Achene).—An achene is a one-seeded indehiscent fruit, the seed of which is free from the pericarp except at one point. The epicarp and mesocarp layers are much reduced and the endocarp exaggerated. The epicarp in surface preparations is of thick-walled, angled cells. The mesocarp consists of a few layers of thin-walled parenchyma cells with traces of fibro-vascular tissue. The endocarp is fibrous.

CELL CONTENTS OF FRUITS

The stored materials in fruits are usually contained within the mesocarp parenchyma cells. Owing to the great diversity in the characters of the different fruits, the cell contents are exceedingly varied and may include starch, sugars, volatile oils, alkaloids, glucosides and calcium oxalate crystals. The various pigments in fruits are classified as protoplasmic cell contents.

FUNCTIONS OF FRUIT TISSUES

The functions of the different structures present in fruits may be summarized as follows:

Covering tissues........ { Epidermis,
Trichomes.

Supporting tissues...... { Stone cells,
Bast fibers (traces),
Wood fibers (traces),
Collenchyma.

Absorbing tissues........Stomata (rarely active).

Conducting tissues...... { Ducts,
Latex tubes.

Assimilating and
secreting tissues...... { Secretion cells,
Glandular hairs.

Storage tissues......... { Parenchyma,
Latex tubes.

CHAPTER XVIII

SEED STRUCTURE

A SEED is a fertilized ovule and consists of coats, nourishing materials and an embryo. The coats are usually two in number, the *testa* or outer and the *tegmen* or inner coat, although a third coat or *aril* may be developed outside the testa. The coats may be merged in such a manner that their structure is difficult to discern. The embryo, or miniature plant, contains or is surrounded by a store of nourishment termed *endosperm*. The term *perisperm* is applied to portions of the nucellus of the ovule, when these persist in the seed. A certain amount of nourishing material may be stored in the nucellus. The tissues present in a seed, in the order of their position, beginning with the outermost, are as follows:

1. Epidermis or testa,
2. Inner epidermis or tegmen,
3. Endosperm tissues,
4. Embryo tissues.

It is difficult to give general descriptions of the cellular elements of seeds for, like fruits, they vary so greatly that it is impossible to select any one seed as a type. In the following notes two types of seeds will be described. In the first type, of which wheat and nux vomica are selected as representatives, the nourishment or endosperm is stored outside of the

embryo and such seeds are termed *albuminous*. In the second type, of which mustard seed and physostigma are examples, the nourishing material or endosperm is stored within the embryo, and these are termed exalbuminous seeds.

WHEAT

The structural elements present in wheat are found with but slight variations in most of the grains. The wheat grain is a fruit, possessing exocarp, mesocarp and endocarp layers, but the major portion of the grain is the seed. The seed is in close proximity to the endocarp, and some of the tissues of the latter layer may be present in wheat flour.

Tissues of the Testa and Tegmen.—These elements are usually closely adherent and each consists of a single layer of cells (Plate 83). On transverse view, the individual e ements appear as small, elongated, rectangular, thick-walled cells in close contact with a thin layer of tissue which rarely shows definite cell structure, and which represents the tegmen. In powdered materials these tissues are seen on surface view and consist of long, thin-walled, slightly colored angled cells of irregular shape. The testa and tegmen cells usually extend to right angles to each other as may be plainly seen in surface preparations.

Endosperm Tissues.—Remnants of a perisperm layer usually occur in the seeds of grains; but the tissues are so reduced that they appear as a thin membrane between the tegmen and the endosperm. The cellular structure of this perisperm can only be distinguished in surface preparations. In transverse sections the outermost layer of the endosperm will
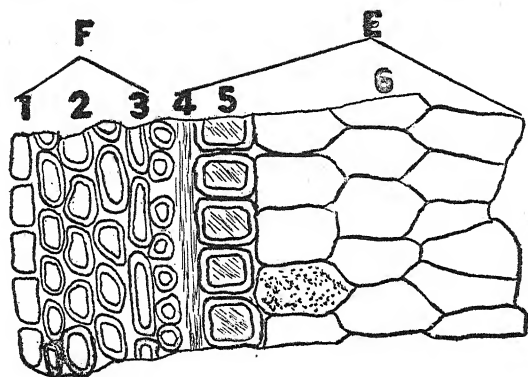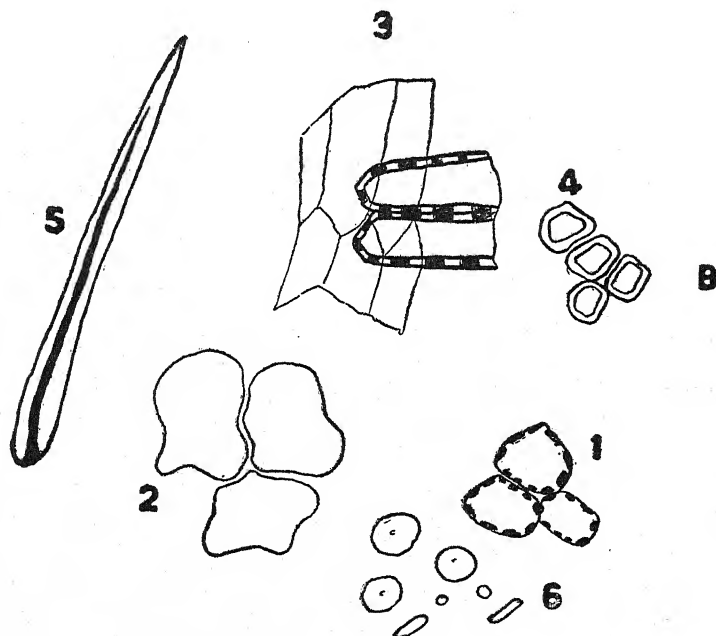
PLATE 83.—Seed Structure.

A. Transverse Section, Wheat. *F.* Pericarp or fruit layers. 1. Epicarp. 2. Mesocarp. 3. Endocarp. *E.* Seed Tissues. 4. Seed coats consolidated with perisperm layer. 5. Endosperm, aleurone layer. 6. Endosperm, parenchyma containing starch.

B. Powdered Wheat. 1. Epicarp tissue. 2. Perisperm tissue. 3. Seed coat tissue. 4. Aleurone cells of the endosperm. 5. Unicellular trichome from epicarp. 6. Starch.

be apparent as regularly arranged, square or rectangular, thick-walled cells (Plate 83), containing darker contents than the cells more deeply located. These cells contain aleurone and are known as *aleurone* or *gluten* cells. The remaining endosperm tissues consist of large, thin-walled parenchyma cells filled with starch. In powdered materials the perisperm layer appears as a thin membrane composed of large, thin, wavy-walled cells. The aleurone cells are apparent in sectional and surface views. On sectional view they are square and usually occur in strips of five to ten adhering cells (Plate 83). On surface view they appear as circular or polygonal forms, with thick white walls and gray contents. The endosperm parenchyma is apparent as irregularly polygonal and circular thin-walled cells, filled with starch and seldom present in unbroken condition.

Embryo Tissues.—The embryo, or germ, of wheat is a small body at one end of the grain. It consists of a large cotyledon, or seed leaf, which encircles the other parts of the embryo. The embryo is composed of small parenchymatic cells with rudiments of fibrovascular tissue. In the milling of flour the embryo is removed from the grain, as, owing to the fat contained, it imparts an unpleasant taste to the product.

NUX VOMICA

This seed occurs entirely free from fruit tissues, and the arrangement of tissues is very similar to that of other seeds possessing a hard, bony endosperm.

Tissues of Testa and Tegmen.—The epidermis of this seed consists of a single layer of thick-walled

porous cells, the free surfaces of which give rise to
extremely long, unicellular trichomes. In sectional
view the epidermal cells are rectangular, and in surface
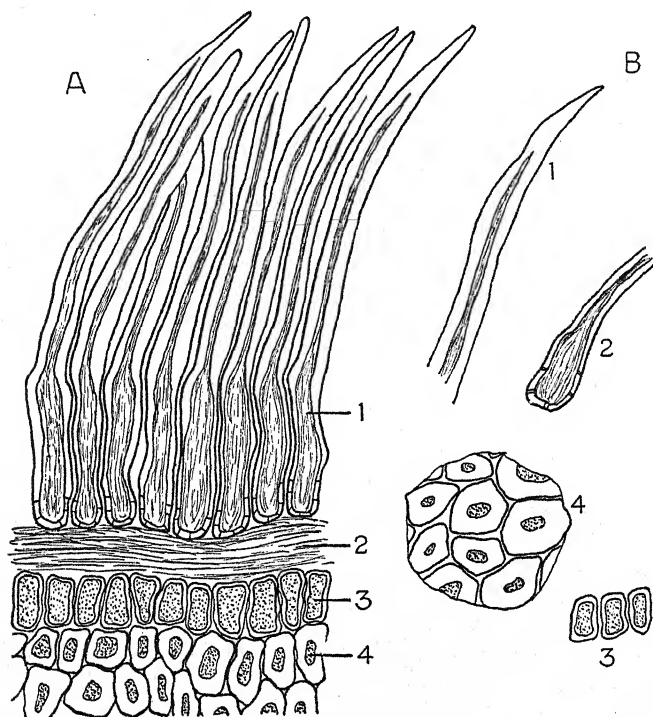
PLATE 84.—Seed Structure.

A. Transverse Section of Nux Vomica Seed. 1. Trichomes from porous cells of
testa. 2. Tegmen zone. 3. Outer layer of endosperm cells. 4. Endosperm cells.
B. Powdered Nux Vomica Seed. 1. Trichomes. 2. Porous testa cell with base
of trichome. 3. Outer endosperm cells. 4. Endosperm cells with thickened walls.

preparations they appear in irregular forms closely
fitted together. The inner epidermal layer consists
of brownish thin-walled cells of a parenchymatic type.
This layer is usually difficult of distinction, owing to
the deep coloration and the collapsed state of the cells.

In powdered materials the trichomes are the most prominent element.

Endosperm Tissues.—The cells of the outer layers of the endosperm are rectangular to radially elongated, with moderately thick walls and containing brownish granular contents. The endosperm cells from the inner portion of the seed are more angled in form and with thicker walls. The walls of these cells are of reserve cellulose and the contents include aleurone and oil.

Embryo Tissues.—The embryo is placed between the halves of the seed endosperm and, even to the unaided eye, shows a high degree of differentiation. The cotyledons or seed leaves are membranous, and upon microscopical examination will show typical leaf structures, excepting chlorophyll. The plumule or stem shows the beginnings of differentiation into the three tissue zones—dermatogen, periblem and plerom, with traces of fibro-vascular elements in the latter.

BLACK MUSTARD

The structural elements present in this seed occur with a fair degree of uniformity in other exalbuminous seeds with membranous seed coats.

Tissues of the Testa and Tegmen.—These tissues are arranged in four layers in the following order, epidermal mucilage cells, subepidermal cells, stone cells and pigment cells. Seen in sectional view (Plate 85), the outer epidermal cells appear as long rectangular forms with slightly thickened walls. The subepidermal cells are large, thin-walled, and very irregular in form, and contain substances which form a mucilage upon contact with water. The swelling of
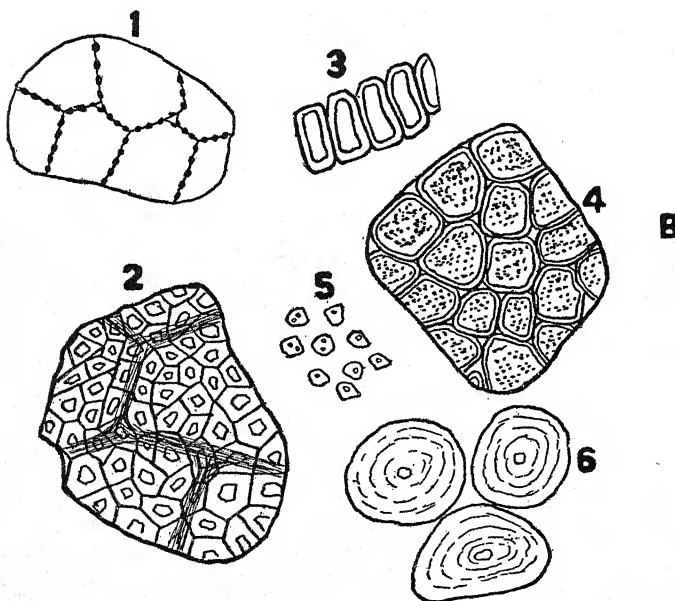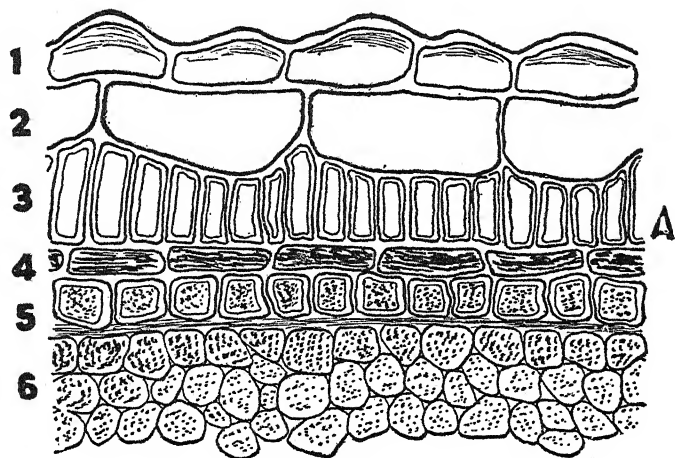
PLATE 85.—Seed Structure.

A. Transverse Section, Black Mustard Seed. 1. Epidermal layer of testa. 2. Subepidermal layer of testa. 3. Palisade layer of testa. 4. Pigment layer or tegmen. 5. Endosperm. 6. Parenchyma of cotyledon containing aleurone.

B. Powdered Black Mustard Seed. 1. Epidermal cells. 2. Subepidermal and Palisade layers consolidated. 3. Palisade cells. 4. Parenchyma of cotyledon. 5. Aleurone grains. 6. Mucilage cells.

the cells which occurs during the formation of this mucilage tends to rupture the seed coat, thus permitting egress of the embryo root. The gums contained in the subepidermal layers of seeds also favor rapid germination by attracting water from the earth. The stone cells are small and of varying lengths, arranged so that the short side of the cell is toward the epidermal layers. These stone cells are termed the palisade layer. The pigment cells are long and narrow, and are so arranged that their long sides are toward the palisade layer. In powdered mustard these tissues are usually seen on surface view, although small fragments of seed coat tissue may be apparent on sectional view. The epidermis (Plate 85) is brown and composed of large, polygonal cells with beaded walls. The subepidermal tissue is usually adherent to the palisade layer and is visible as large polygonal cells of indistinct form in fragments of the palisade tissue. The palisade tissue is composed of small, thick-walled, angled stone cells. The pigment layer consists of large, angled cells of dark color, usually adherent to the palisade tissue.

Endosperm Tissues.—The endosperm of mustard is contained within the cotyledons or miniature leaves of the embryo. The cotyledons are covered by a thin epidermal layer of cutinized cells. The endosperm cells (Plate 85), are angled or irregularly circular in form with slightly thickened white walls. In powdered mustard the endosperm cells usually appear in masses composed of irregularly circular, white-walled cells containing a grayish aleurone.

Embryo Tissues.—The cotyledons of the mustard embryo may show distinct signs of tissue differentiation.

A rudimentary leaf epidermis and traces of palisade cells are present; and the fibro-vascular elements are represented in the stem portion of the embryo.

PHYSOSTIGMA

This seed, although of exalbuminous type, differs from the mustard seed in possessing a hard and tough seed coat. The stone-cell or palisade layer shows greater development and thus affords greater protection to the embryo.

Tissues of Testa and Tegmen.—In sections the testa consists of an outer layer of radially elongated, fiber-like cells. These cells are white in color, with thick walls and a reddish-brown content. Immediately beneath this outer layer is a zone of irregular, white-walled cells, with rather large cavities filled with a reddish-brown substance. It is probable that this is a subepidermal layer. The tegmen consists of several layers of small, thin-walled, dark-colored cells, among which small amounts of fibro-vascular tissue may be present. In powdered materials the cells of the two outer layers are very prominent, but the inner seed coat is hardly distinguishable.

Endosperm Tissues.—The endosperm of this seed is contained in the large cotyledons and the stored material is starch. The cotyledons are mainly of parenchymatic tissue with a thin outer layer corresponding to an epidermis. In powdered physostigma these parenchyma cells are so disintegrated that but slight traces of the endosperm parenchyma are apparent. The large and characteristic starch grains contained in the endosperm parenchyma cells are very numerous.
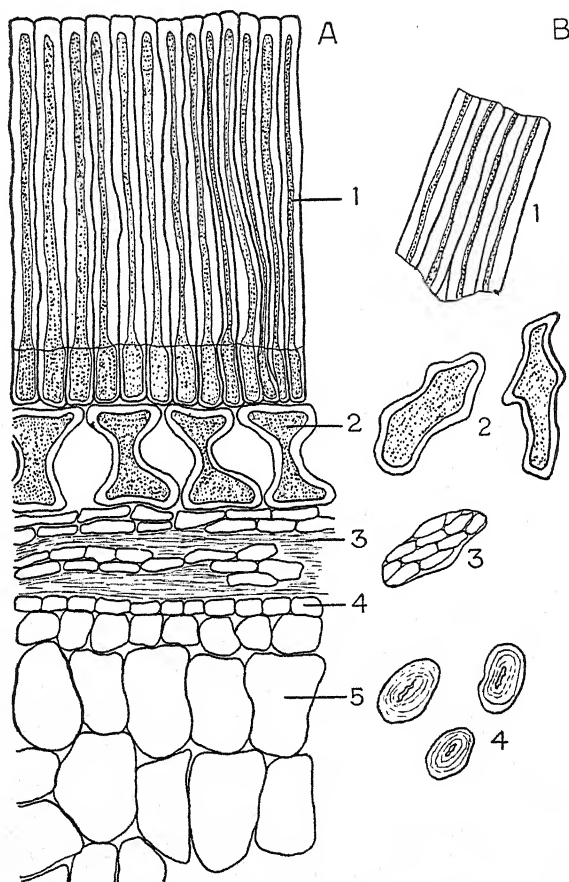
PLATE 86.—Seed Structure.

A. Transverse Section of Physostigma Seed.   1. Palisade cells of testa.   2. Subepidermal cells of testa.   3. Parenchymatic cells of tegmen.   4. Epidermal cells of cotyledon.   5. Seed parenchyma cells.

B. Powdered Physostigma Seed.   1. Palisade cells.   2. Subepidermal cells. 3. Tegmen cells.   4. Starch grains.

Embryo Tissues.—The cotyledons show a lesser degree of differentiation than in mustard seed. The stem portion shows the three tissue zones, with traces of fibro-vascular tissue in the plerom.

CELL CONTENTS OF SEEDS

The cell contents of seeds are contained within the parenchyma of the endosperm. These contents are exceedingly varied and may include starch, aleurone, fixed oils, volatile oils, alkaloids and glucosides. Aleurone usually occurs in seeds containing fixed oil; but starch is rarely present in seeds containing these substances.

FUNCTIONS OF SEED TISSUES

The functions of the different structures present in seeds may be summarized as follows:

Covering tissues..........$\left\{\begin{array}{l}\text{Epidermis,}\\ \text{Stone cells.}\end{array}\right.$

Supporting tissues........$\left\{\begin{array}{l}\text{Stone cells,}\\ \text{Fibers (rarely).}\end{array}\right.$

Storage tissues...........$\left\{\begin{array}{l}\text{Parenchyma,}\\ \text{Collenchyma.}\end{array}\right.$

CHAPTER XIX

MICROSCOPE ACCESSORIES

MECHANICAL STAGE

THE device known as the mechanical stage is essential in work requiring the systematic examination of several fields or all parts of the specimen. It is used in the quantitative estimation of the different types of cells in blood samples; in the examination of urinary sediments, and in bacterial, mold and spore counts. The more complicated types of microscopes are provided with mechanical stages which are permanently attached to the instrument. This construction presents the advantages of rigidity and fixed position. However, these fixed mechanical stages are often inconvenient and are liable to corrosion if temporary mounts are frequently used. To overcome these faults many manufacturers supply an extra, or plain, stage, which is interchangeable and can be substituted for the fixed mechanical stage. The attachable mechanical stage serves all purposes and is the type generally used. It consists of a vertical frame firmly attached to a horizontal frame, the latter part of the apparatus being equipped with slide clips. Movement of the frames in vertical and horizontal directions is secured by milled wheels attached to geared pinions, the teeth of which are fitted to racks upon the frames. Each frame bears a graduated

scale which, in combination with a vernier, reads
to 0.1 millimeter. After the accuracy of these scales
is checked by comparison with a stage micrometer,
they may be used for the approximate measurement
of fairly large objects. The diameter of the micro-
scope field with different combinations of objectives
and oculars may be ascertained by placing an object
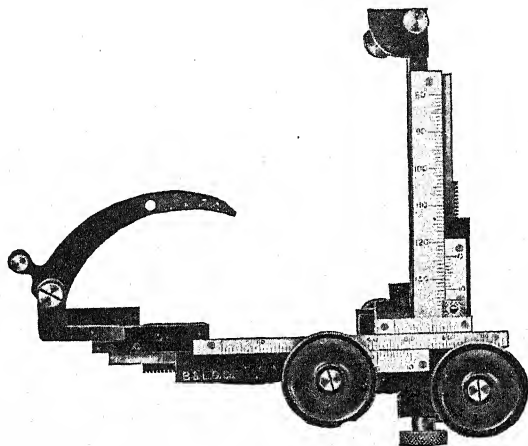
FIG. 87.—Mechanical Stage.
(Bausch & Lomb.)

at the edge of the field, noting the scale reading, then
moving the object to the opposite edge of the field
and making a second scale reading. The difference
in readings will give the diameter of the field with a
given combination of lenses.

MICROMEASUREMENTS

Occasionally it is necessary to ascertain the dimen-
sions of cell and cell contents. This may be accom-

plished by several methods, but the accessories usually employed for this purpose are the ocular micrometer and the filar micrometer. Ocular micrometers, while not as accurate as the filar types, are less expensive and sufficiently accurate for all but the most critical observations.

Ocular Micrometer.—This apparatus consists of a glass disk of proper size to fit within the ocular mounting and having a graduated scale ruled upon its surface. The number of scale divisions varies; but, as a rule, the fifth and tenth lines are longer than the others. As the value of these scale divisions varies with different microscopes and combinations of lenses, it is necessary to standardize the ruling by comparing it with a stage micrometer scale. The stage micrometer is a slide upon which is etched an accurately ruled scale 1 or 2 millimeters in length, each millimeter being divided into 100 parts.

Standardization of Ocular Micrometers. — In standardizing micrometers the tube length should be adjusted at 160 millimeters. Place the ocular micrometer slip in the ocular so that it rests upon the diaphragm of the latter. Place the stage micrometer on the stage and focus so that the rulings are clear. Move the stage micrometer slide and turn the ocular until the stage and ocular scales correspond and the end line of the ocular scale coincides with a line of the stage micrometer ruling. Count the number of spaces of the stage scale covered by the entire number of ocular micrometer lines. Divide the number of stage micrometer lines covered by the total number of ocular scale divisions. The quotient equals the value in millimeters of each line of the ocular scale.

Example.

100 divisions of ocular scale cover 56 of the stage scale.
1 division of stage scale equals 0.01 millimeter.
56 divisions of stage scale equals 0.56 millimeter.
100 divisions of ocular scale equals 0.56 millimeter.
1 division of ocular scale equals 0.0056 millimeter.

In standardizing ocular micrometers with high-power objectives it often happens that the end lines of the ocular micrometer fall between lines of the stage micrometer. This difficulty may be overcome by
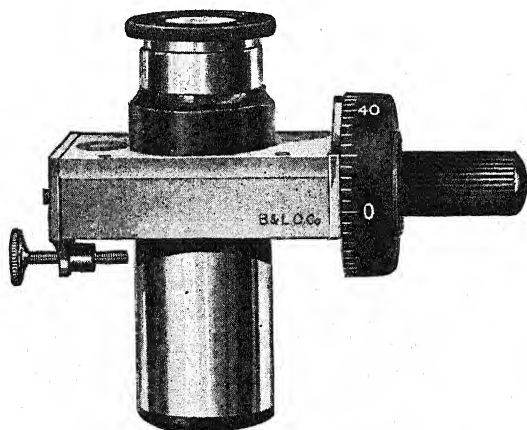
FIG. 88.—Filar Micrometer.
(Bausch & Lomb.)

making slight changes in the tube length until the lines of both micrometers coincide. Micrometer values must be established for each combination of objectives and oculars, and the tube length must be constant for each valuation. Record should be made of the micrometer values and tube lengths with the different combinations.

Filar Micrometers.—This type of micrometer consists of an ocular fitted with a movable scale or hair line, which, when a graduated drum is revolved, traverses a fixed scale. The micrometer ocular replaces the ocular of the microscope, and the mounting is usually provided with a set screw to prevent the apparatus turning in the draw tube. The scale and drum rulings of a filar micrometer must be standardized against a stage micrometer in the manner described in the preceding section. Filar micrometers are much more accurate than the disk types; but as they are integral parts of the ocular, one is limited, in using them, to the single ocular in which the scale is mounted. Disk micrometers may be transferred from one ocular to another of different power, and one scale may be standardized for use with a wide range of oculars.

The unit of measurement in microscopy is the micron (μ), which is equivalent to 0.001 millimeter (1/2500 inch), and the dimensions of microscopic objects are expressed in microns.

CAMERA LUCIDA

This accessory is used in the preparation of sketches or drawings of microscopic objects. The essential parts of the apparatus are a prism and a mirror. The reflecting apparatus is so placed that the observer may see the specimen and the drawing surface at the same time. In brief, the instrument serves to combine the image produced by the microscope with a view of the drawing surface. One of the reflecting elements is a cube consisting of two triangular glass prisms separated from each other by a silvered surface.

This silvered surface shows a small perforation in line with the optic axis of the microscope lenses, thus permitting passage of light rays through the
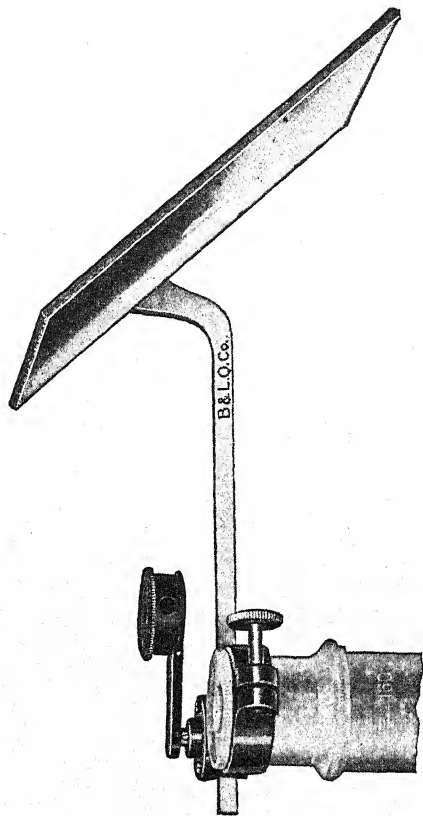
FIG. 89.—Camera Lucida, Adjustable Mirror Type. (Bausch & Lomb.)

silvered surface to the eye. The other reflecting element is an adjustable mirror supported on a bar extending at right angles to the optic axis of the microscope. This mirror reflects the image of the drawing paper to the silvered surface of the glass prism, which

in turn acts as a mirror and transmits the view of the drawing paper to the eye. At the same time views of the microscope image are apparent through the perforation in the silvered surface.

The mirror should be adjusted at an angle of 45 degrees from the optic axis, and the drawing surface should be horizontal and level. Where these adjustments interfere with the proper projection of the image the angle of the mirror may be slightly reduced. If the angle of the mirror is varied from 45 degrees, the outer edge of the drawing board must be tilted toward the microscope, the drawing surface
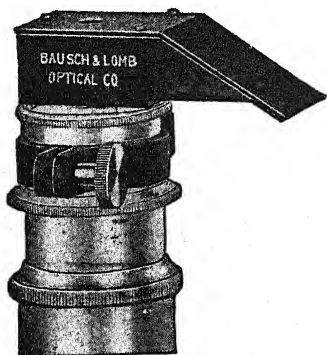
FIG. 90.—Camera Lucida, Fixed Mirror Type.
(Bausch & Lomb.)

being raised twice as many degrees as the mirror is depressed. Certain types of instruments are equipped with slips of slightly darkened glass which are interposed between the prism and the mirror. As the drawing surface is usually white it reflects an excess of light, and this condition is overcome by use of the darkened glass disks.

POLARIZING APPARATUS

Although the polarizing microscope is extensively used in chemical microscopy, comparatively little has been done in the adaptation of the apparatus to the field of histology. In order to comprehend the working of the polarizing apparatus one must

keep in mind certain physical concepts of light. Ordinary light rays, emanating from the sun or other sources, are in the form of innumerable waves or vibrations of various and constantly changing direction. Certain transparent and translucent materials possess the property of separating light waves and permitting only those which are parallel to a given plane to pass. This phenomenon is termed *polarization*, and the light rays emerging from these materials constitute polarized light. Calcite or Iceland spar is the material used in the construction of polarizing apparatus. The calcite is cut into long rhombic prisms (*nicol prisms*), which are then cut obliquely and cemented together with Canada balsam. These prisms transmit certain light rays with but slight refraction, so that they pass through both parts of the nicol and are visible. Other light rays are refracted to such an extent that, upon striking the cemented surface between the prisms, they are totally reflected and are not visible at the upper surface of the nicol. The totally refracted light waves are termed *ordinary rays*, while the waves which are so slightly refracted that they pass through both prisms are termed *extraordinary rays*.

A complete polarizing apparatus consists of two nicol prisms, the *analyser*, which is placed below the object under observation, and the *polarizer*, which is placed above it. The mountings of both polarizer and analyser may be graduated, and either or both may be so arranged as to rotate easily. A graduated rotating stage provided with centering devices is necessary in all but the most superficial observations. Upon rotating the polarizer one observes that the

field becomes alternately light and dark. It will be further noted that, starting with a field of maximum darkness, rotation through an angle of 90 degrees will be required to secure a field showing maximum brightness. If the prisms are so placed that the light rays passing through them are parallel, a light field results, and the nicols are said to be in the *uncrossed position*. If one of the prisms is rotated so that the light rays passing through it are not parallel with those passing through the other prism, a dark field results, and the nicols are said to be in the *crossed position*. If the mounting of the polarizer is graduated the prism should be so arranged that fields of maximum darkness are visible at 90 and at 270 degrees.

The polarizing apparatus is used in the determination of optical and crystallographic properties of different substances. For these observations the prisms should be set in a crossed position. All transparent and translucent materials may be classified according to the characters they exhibit when viewed between crossed nicols. *Isotropic* or singly refracting substances do not polarize or change the direction of light rays, and therefore show no change when rotated between crossed prisms. Crystals of the isometric type and most amorphous substances are isotropic. The isotropic vegetable tissues and cell contents include cork, epidermis, parenchyma, inulin and oil globules. *Anisotropic* or doubly refracting substances polarize or change the direction of light rays and therefore become alternately light and dark upon rotation between crossed nicols. Most crystalline chemical substances, excepting those of isometric form, are anisotropic. The vegetable tis-

sues and cell contents which act similarly to aniso-
tropic substances include fibers, vessels, calcium
oxalate crystals, starch and mucilage deposits.

In certain anisotropic substances the property
of double refraction is barely apparent and only

FIG. 91.—Adjustable Microscope Lamp.
(Bausch & Lomb.)

neutral gray tints are visible upon rotation.   In these
instances a thin plate of selenite is interposed between
analyser and polarizer.   The selenite disk should
be so placed that its plane of vibration is at an angle
of 45 degrees to, or midway between, the planes of
vibration of the crossed prisms.   The field will now
appear purple-red in color, and the change caused by

rotating weakly polarizing substances will be more apparent because of the greater contrast.

MICROSCOPE LAMPS

Gas or electricity may be used as a source of light for microscopic illumination. To obtain satisfactory illumination with gas, it is best to use burners equipped with inverted mantels. Burners of this type give a fairly steady light, free from shadows cast by fixtures or other interfering objects. The ordinary
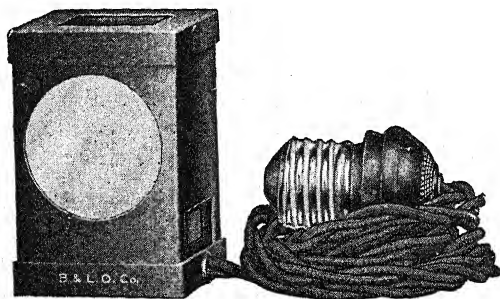
Fig. 92.—Microscope Lamp.
15-watt concentrated filament.
(Bausch & Lomb.)

open flame gas burners are unsatisfactory because of the unsteadiness of the light.

The electric illuminating devices include arc and filament lamps. The electric arc furnishes the most intense illumination, and is especially useful in microphotography, owing to the actinic quality of this light. Arc lamps can only be used in conjunction with a rheostat or current control device, and one must look to adjustment of the carbons at frequent intervals.

While the light from filament lamps is not as intense as that of the electric arc, it is sufficient for practically all purposes. Low-voltage types of filament lamps require a rheostat or resistance device. High-voltage lamps are more convenient in that they can be directly connected with the source of current. Condensing
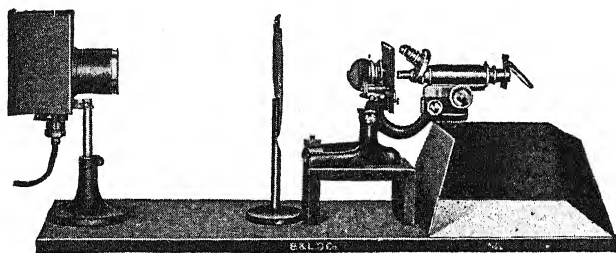
Fig. 93.—Drawing Apparatus with Adjustable Lamp.
(Bausch & Lomb.)

lenses are usually interposed between the lamp and the microscope, so that the light rays may be concentrated within a small area. In long-continued observations, electric illumination is often more desirable than daylight because of its constant intensity and the ease with which it may be regulated.

APPENDIX

FORMULÆ

ACID ALCOHOL:

      Conc. Hydrochloric Acid.................   0.5 mil

      Alcohol, to make....................... 100.0 mils

ALCOHOL, GLYCERIN, WATER MIXTURE:

   Used as a temporary mounting medium and for maceration of materials.

   Alcohol,

   Glycerin,

   Distilled Water, equal parts by volume.

ALKANNIN SOLUTION:

   Formula A

      Alkanet root.......................... 20 grams

      Water................................ 100 mils

   Macerate for several days. Filter and dilute with equal parts of water before using.

   Formula B

   Alkannin dissolved in absolute alcohol. Add equal parts of water and filter before using.

ALPHA NAPHTHOL:

      Alpha Naphthol........................ 20 grams

      Alcohol, to make...................... 100 mils

ANILINE DYES:

      Saturated alcoholic solution of dye........ 5 mils

      Distilled Water, to make................ 100 mils

ANILINE WATER SAFRANNIN:

    1. Aniline water.

   Saturate distilled water with aniline by vigorous shaking.

    2. Safranin, saturated alcoholic solution.

   Mix equal volumes of 1 and 2 and filter before using. This reagent should be freshly prepared.

ALTMAN'S PICRIC ACID:

    Picric Acid.............................. 8 grams

    Alcohol, to make........................ 100 mils

Dilute with two volumes of water before using.

BOUIN'S FLUID:

    Fixing agent for animal and vegetable materials.

        Picric acid, saturated alcoholic solution..... 200 mils

        Formalin.................................... 80 mils

        Glacial Acetic Acid...................... 15 mils

Time required is two to five days. After fixing, wash the specimens with 50 per cent alcohol until the latter fails to show yellow coloration.

CHROMIC ACID:

    Chromic Acid......................... 1 gram

    Distilled Water, to make................ 100 mils

CHLORAL HYDRATE:

    Chloral Hydrate........................ 80 grams

    Distilled Water......................... 50 mils

CLEANING FLUIDS FOR GLASSWARE AND SLIDES:

    Conc. Nitric Acid...................... 1 part

    Conc. Hydrochloric Acid................ 4 parts

Uncovered glass containers must be used for this mixture and, owing to the corrosive fumes liberated, vessels containing glassware and cleaning fluid must be kept under a hood or in the open air. After immersion for a few hours the glassware should be well washed in water.

        Potassium dichromate.................... 20 grams

        Water.................................. 100 mils

        Conc. Sulphuric Acid.................... 100 mils

Dissolve the dichromate in the water, add the acid cautiously and in small portions, cooling the mixture between each addition. The mixture must be stored in glass containers and may be repeatedly used until the color changes. Immersion for a few hours followed by washing in water will serve to clean slides and covers.

CLEARING AGENTS:

    Clove, Lemon, Turpentine, Bergamot and Sassafras Oils,

    Liquefied Phenol,

    Xylol,

    Chloroform,

    Canada Balsam in Xylol.

Cuprammonia, (Ammoniacal Copper) (Schweitzer's Reagent) (Cuoxam):

Formula A

    Copper Sulphate.............15 per cent aqueous solution
    Sodium Hydroxide.......... 3 per cent aqueous solution

Add slight excess of the hydroxide solution to the copper sulphate solution. Wash the precipitate by decantation and dissolve it in concentrated ammonia water.

Formula B

Cover small pieces of metallic copper with 15 per cent ammonia water. Allow the mixture to stand in an open vessel for several days. Filter through glass wool. This reagent should dissolve cotton immediately and completely.

Cupric Acetate:

    Cupric Acetate.......................... 15 grams
    Distilled Water, to make................ 100 mils

Delafield's Hematoxylin:

    Hematoxylin........................... 4 grams
    Alcohol............................... 25 mils
    Ammonia Alum......................... 50 grams
    Glycerin.............................. 100 mils
    Methyl Alcohol........................ 100 mils
    Distilled Water....................... 400 mils

Dissolve the hematoxylin in the alcohol and the alum in the water. Mix these solutions and allow to stand exposed to light and air for five days. Filter, add the glycerin and methyl alcohol and allow to stand exposed to light for five days. Finally filter and store for use. This stain improves upon keeping.

Fehling's Solution (Alkaline Cupric Tartrate):

Alkaline Tartrate Half.

    Rochelle Salt......................... 17.3 grams
    Sodium Hydroxide..................... 5.0 grams
    Distilled Water, to make.............. 50.0 mils

Copper Half.

    Copper Sulphate...................... 3.4 grams
    Distilled Water, to make.............. 50.0 mils

Mix equal parts of each solution.

FERRIC CHLORIDE:

    Ferric Chloride.......................... 1.0 gram
    Distilled Water.......................... 100.0 mils

FLEMMING'S SOLUTION (CHROMO-ACETO-OSMIC ACID):

Weak solution.

    Chromic Acid (1 per cent aqueous solution).... 25 cc.
    Osmic Acid (1 per cent aqueous solution)...... 10 cc.
    Acetic Acid (1 per cent aqueous solution)... 10 cc.
    Water................................... 55 cc.

Strong solution.

    Chromic Acid (10 per cent aqueous solution)... 7.5 cc.
    Osmic Acid (1 per cent aqueous solution)...... 40.0 cc.
    Glacial Acetic Acid....................... 5.0 cc.
    Water................................... 47.5 cc.

As this mixture deteriorates, only small quantities should be prepared and it should not be exposed to light.

FIXING FLUIDS:

Refer to Bouin's Fluid, Chromic Acid, Formalin, Mercuric Chloride, Picric Acid, Picric Sulphuric Acid, Zenker's Fluid and Flemming's Solution.

FORMALIN:

Used as a preservative and fixing fluid for animal and vegetable tissues.

    Formalin.................................. 10 mils
    Water.................................... 90 mils

Time required for fixing is two to five days. This solution is not always thorough in its fixing action and sometimes interferes with staining.

GLYCERIN JELLY:

    Gelatin................................... 10 grams
    Glycerin.................................. 70 mils
    Water.................................... 60 mils
    Liquefied Phenol.......................... 1 mil

Soak the gelatin in water until soft; add the glycerin and gently warm until thoroughly dissolved. Add the phenol and filter through glass wool.

IODINE POTASSIUM IODIDE (DILUTE LUGOL'S SOLUTION):

    Iodine..................................... 0.35 gram
    Potassium Iodide........................ 1.50 grams
    Distilled Water, to make................ 100.00 mils

MERCURIC POTASSIUM IODIDE (MAYER'S REAGENT):

    Mercuric Chloride....................... 1.35 grams
    Potassium Iodide........................ 5.00 grams

Dissolve the mercuric chloride in 60 mils of distilled water and the iodide in 10 mils of distilled water. Mix these solutions and add water to make 100 mils.

MACERATING MIXTURE:

Refer to Schulze's Macerating Mixture.

MERCURIC CHLORIDE:

    Mercuric Chloride....................... 0.5 gram
    Alcohol or Distilled Water, to make........ 100.0 mils

MILLON'S REAGENT:

    Mercury................................ 10 mils
    Conc. Nitric Acid....................... 90 mils

Dissolve the mercury in the acid and dilute this solution with an equal volume of water before using. This reagent easily decomposes and but small quantities should be prepared.

PHENYLHYDRAZINE MIXTURE:

    Phenylhydrazine Hydrochloride............ 3 grams
    Sodium Acetate......................... 4 grams
    Distilled Water........................ 10 mils

PHLOROGLUCIN:

    Phloroglucin........................... 0.1 gram
    Alcohol................................ 10.0 mils

This reagent decomposes upon standing.

PICRIC ACID:

Saturated alcoholic or aqueous solution.

PICRIC SULPHURIC ACID:

    Picric Acid, saturated aqueous solution..... 98 mils
    Concentrated Sulphuric Acid............. 2 mils

PYROGALLOL:

Pyrogallol	0.1 gram
Alcohol	5.0 mils
Conc. Hydrochloric Acid	5.0 mils

This reagent decomposes upon standing.

SCHULZE'S MACERATING MIXTURE:

Concentrated Nitric Acid with the addition of small amounts of Potassium Chlorate.

ZENKER'S FLUID:

Used as a fixing agent for animal tissues.

Mercuric Chloride	5 grams
Potassium Dichromate	1 gram
Sodium Sulphate	1 gram
Water	100 mils

Add 5 mils Glacial Acetic Acid before using.

Time required for fixation is from one to three days. After fixing, the specimen should be washed in running water for at least twelve hours. Transfer to 50 per cent alcohol for three hours, then to 65 per cent alcohol and finally to 80 per cent alcohol, to which has been added sufficient concentrated iodine potassium iodide solution to give the alcohol a dark-brown color. The purpose of the iodine is to remove traces of the mercurial salts which tend to precipitate in the cells. If the iodine alcohol mixture is decolorized, it must be renewed. After two or three days the material is transferred to fresh 80 per cent alcohol, which is changed frequently until all iodine is removed from the specimen.

ZINC CHLORIDIDE:

Zinc Chloride	25 grams
Potassium Iodide	8 grams
Iodine	1 gram
Distilled Water, to make	15 mils

Dissolve the zinc chloride and the potassium iodide in the water, and add as much iodine as can be dissolved.

TABLE OF MAGNIFICATIONS

(Adapted from Catalogues of Bausch & Lomb, Leitz and Spencer.)

OBJECTIVES.

OCULARS.

OBJECTIVES.

| American (diameters) | 2.5x | 5x | 7.5x | 10x | 12.5x | 15x | 20x | |
| Continental (empirical) | 1 | 2 | 3 | 4 | 5 | 6 | 8 | |
English (inches)	2	1⅗	1⅕	1	⅘	¾	½	
mm.								mm.
32	10	20	30	40	50	60	80	32
16	25	50	75	100	125	150	200	16
8	50	100	150	200	250	300	400	8
4	110	215	320	430	560	660	880	4
3	144	285	420	570	740	900	1200	3
1.9	238	475	720	950	1260	1425	1900	1.9
1.5	410	545	870	1090	1310	1635	2180	1.5

TABLE OF OBJECTIVES

Metric (millimeters)......	32	25	16	12	8	5	4	3	2	1	1.9	1.5
English (inches).........	1⅓	1	⅔	½	⅓	⅕	⅙	⅛	¼	1/10	½	1/15
Continental (numbers).....	1	2	3	4	5	6	7	8	9	1/10	1/12	1/15
Numerical Aperture........	.08	.25	.25	.40	.50	.70	.85	.85	.87	1.20	1.30	1.30
Working Distance (mm.)....	38	9.5	7.0	1.8	1.6	1.5	0.3	0.2	.16	.11	0.15	0.13
Real Field Diameter (mm.).	5.5	3.3	2.1	1.4	1.0	0.5	0.4	0.3	.24	.23	0.2	0.14
Objective Magnification...	4	6	10	15	20	36	43	57	70	85	95	109

COVER–SLIPS

COMMON SIZES, THICKNESSES, SHAPES AND DESIGNATIONS

(Adapted from Catalogue, Spencer Lens Co.)

Desig-nation.	Thickness.	Shapes.	Sizes Manufactured.
No. 0	less than 0.13 mm.	Squares, Circles	13, 16, 18, 22, 25 mm.
No. 1	0.13 to 0.17 mm.	Squares, Circles	13, 16, 18, 22, 25 mm.
No. 2	0.17 to 0.25 mm.	Squares, Circles	13, 16, 18, 22, 25 mm.
No. 3	0.25 to 0.50 mm.	Squares, Circles	13, 16, 18, 22, 25 mm.

APPROXIMATE NUMBER OF COVERS PER OUNCE

Circles.	13 mm.	16 mm.	18 mm.	22 mm.	25 mm.
No. 0	855	550	375	254	214
No. 1	564	362	280	182	142
No. 2	444	286	195	157	112
No. 3	372	240	166	122	93
Squares.					
No. 0	700	450	312	208	176
No. 1	462	296	206	150	116
No. 2	364	234	162	120	92
No. 3	306	196	136	100	76

REFERENCE BOOKS

GENERAL:

    United States Pharmacopœia, X.
    National Formulary, V.
    National Standard Dispensatory; Hare, Caspari, Rusby.
    United States Dispensatory; Wood, Remington, Sadtler.
    The Microscope, Gage.
    Elements of Applied Microscopy, Winslow.
    The Microscope and its Revelations, Carpenter-Dallinger.
    Analytical Microscopy, Wallace.

BOTANY:

    Text Book of Botany; Strassburger, Noll, Schenck and Karsten.
    Manual of Botany, Rusby.
    Applied and Economic Botany, Kraemer.
    Physiological Plant Anatomy, Haberlandt.
    Textbook of General Botany, Holman and Robbins.
    Nature and Development of Plants, Curtis.
    Text Book of Botany, Vol. I, Coulter, Barnes and Cowles.

MICROSCOPY OF FOODS:

    Microscopy of Vegetable Foods, Winton.
    Microscopy of Technical Products, Hanausek-Winton.
    Bacteriology and Mycology of Foods, Tanner.
    Food Microscopy, Clayton.

MICROSCOPY OF DRUGS:

    Scientific and Applied Pharmacognosy, Kraemer.
    Powdered Drugs, Schneider.
    Histology of Medicinal Plans, Mansfield.
    Powdered Drugs, Greenish.
    Pharmacognosy, Youngken.

MICROCHEMISTRY:

    Botanical Microchemical Technic, Zimmerman.
    Elementary Chemical Microscopy, Chamot.
    Microchemistry of Poisons, Wormley.

INDEX

(Figures preceded by an asterisk (*) indicate pages upon which illustrations appear)